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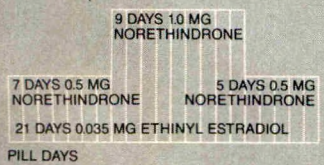


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Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

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THE NATURE OF TRI-NORINYL®

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TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

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ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to non-smoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS–RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A–Use associated with very high risk.
B–Use associated with high risk.
C–Use associated with moderate risk.
D–Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones–estrogenic and progestational agents–during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests *Chlamydia trachomatis* infection, the leading cause of nongonococcal PI.D., is enhanced by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

Rev. 6/86

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

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Contents

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TRANSACTIONS OF THE FORTY-NINTH ANNUAL MEETING OF THE SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

Responsibility, obligation, and duty 521
Leo J. Dunn, M.D.
Richmond, Virginia

A prospective comparative study of the extended nonstress test and the nipple stimulation contraction stress test 531
Lawrence D. Devoe, M.D., John Morrison, M.D., James Martin, Jr., M.D., Sue Palmer, M.D., Rick Martin, M.D., Nancy Searle, B.A., and Martha Arthur, R.N., B.S.N.
Augusta, Georgia, and Jackson, Mississippi

Ovarian prostaglandin synthase: Immunohistochemical localization in the rat 537
Thomas E. Curry, Jr., Ph.D., Anita Malik, B.S., and Martin R. Clark, Ph.D.
Miami, Florida

(Contents continued on page 6A)

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Contents continued from page 5A

Evaluation of the atypical Pap smear 544
D. E. Darnell Jones, M.D., William T. Creasman, M.D., Raymond A. Dombroski, M.D., Samuel S. Lentz, M.D., and John L. Waeltz, M.D.
Greenville and Durham, North Carolina

Regional program for prevention of premature birth in northwestern North Carolina 550
Paul J. Meis, M.D., J. M. Ernest, M.D., Mary Lou Moore, R.N.C., Ph.D., Robert Michielutte, Ph.D., Penny C. Sharp, M.Ed., and Paul A. Buescher, Ph.D.
Winston-Salem and Raleigh, North Carolina

Careful surgical technique can reduce infectious morbidity after cesarean section 557
James B. Lyon, M.D., and A. Cullen Richardson, M.D.
Atlanta, Georgia

Urogynecology: Comments and caveats 563
Henry A. Thiede, M.D., and Vasant D. Saini, Ph.D.
Rochester, New York

Necrotizing fasciitis of the vulva 568
David B. Roberts, M.D.
Columbus, Georgia

Outcomes in patients with unusually high maternal serum α-fetoprotein levels 572
Lewis H. Nelson, M.D., Jeanette Bensen, M.S., and Barbara K. Burton, M.D.
Winston-Salem, North Carolina

Mechanical intestinal obstruction in patients with gynecologic disease: A review of 368 patients 577
Hans-B. Krebs, M.D., and Dean R. Goplerud, M.D.
Richmond, Virginia

Fetal death: Diagnosis and management 583
Roy M. Pitkin, M.D.
Iowa City, Iowa

Another look at the second-assessment procedure for ovarian epithelial carcinoma 590
Donald G. Gallup, M.D., O. Eduardo Talledo, M.D., Michelle R. Dudzinski, M.D., and Keith W. Brown, D.O.
Augusta, Georgia, and Portsmouth, Virginia

Contents

Endometrial metaplasia associated with endometrial adenocarcinoma 597
Willie A. Andersen, M.D., Peyton T. Taylor, Jr., M.D., Robert E. Fechner, M.D., and Jo Ann V. Pinkerton, M.D.
Charlottesville, Virginia

Conservative surgical management of isthmic ectopic pregnancies 604
Harriet O. Smith, M.D., Andrew A. Toledo, M.D., and John D. Thompson, M.D.
Atlanta, Georgia

Simultaneous objective and subjective evaluation of meclofenamate sodium in the treatment of primary dysmenorrhea 611
Roger P. Smith, M.D., and J. Roger Powell, M.D.
Augusta, Georgia, and Urbana, Illinois

Hysterosalpingographic evaluation of tubal patency after ectopic pregnancy 618
Dorothy E. Mitchell, M.D., Helen F. McSwain, M.D., James A. McCarthy, M.D., and Herbert B. Peterson, M.D.
Atlanta, Georgia

Transurethral ultrasound-guided oocyte retrieval for in vitro fertilization 622
Santiago L. Padilla, M.D., William J. Butler, M.D., Jeffrey P. Boldt, Ph.D., Richard H. Reindollar, M.D., Sandra P. T. Tho, M.D., Gerald B. Holzman, M.D., and Paul G. McDonough, M.D.
Augusta, Georgia

TRANSACTIONS OF THE SEVENTH ANNUAL MEETING OF THE SOCIETY OF PERINATAL OBSTETRICIANS

The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores 627
Anthony M. Vintzileos, M.D., Sue Ellen Gaffney, R.N.C., M.S.N., Lauren M. Salinger, R.N.C., M.S.N., Vasilios G. Kontopoulos, M.D., Winston A. Campbell, M.D., and David J. Nochimson, M.D.
Farmington, Connecticut

Fetal cerebellar growth unaffected by intrauterine growth retardation: A new parameter for prenatal diagnosis 632
E. Albert Reece, M.D., Israel Goldstein, M.D., Gianluigi Pilu, M.D., and John C. Hobbins, M.D.
New Haven, Connecticut, and Bologna, Italy

A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor 638
Jay D. Iams, M.D., Francee F. Johnson, R.N., B.S.N., Richard W. O'Shaughnessy, M.D., and Lou C. West, R.N., B.S.N.
Columbus, Ohio

(Contents continued on page 9A)

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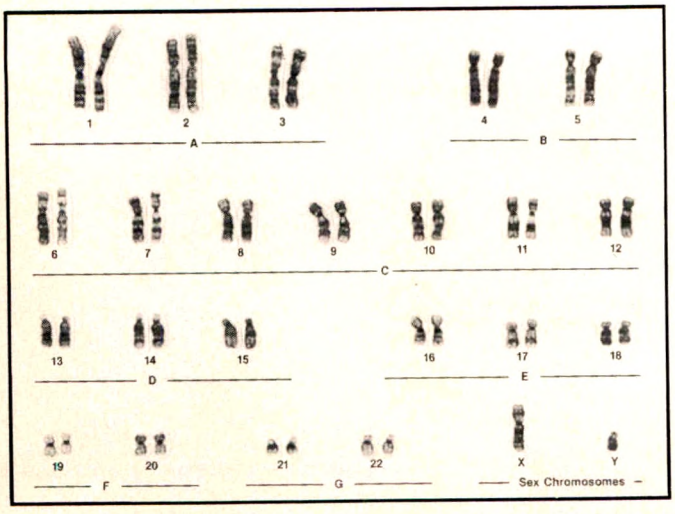
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Contents continued from page 7A

Chronic oral terbutaline tocolytic therapy is associated with maternal glucose intolerance 644
Elliott K. Main, M.D., Denise M. Main, M.D., and Steven G. Gabbe, M.D.
Philadelphia, Pennsylvania

Fetal echocardiographic screening for congenital heart disease: The importance of the four-chamber view 648
Joshua A. Copel, M.D., Gianluigi Pilu, M.D., Jacqueline Green, M.S., R.D.M.S., John C. Hobbins, M.D., and Charles S. Kleinman, M.D.
New Haven, Connecticut

Ultrasound evaluation of tachycardia-induced hydrops in the fetal lamb 655
C. Nimrod, M.B., D. Davies, R.D.M.S., J. Harder, M.D., S. Iwanicki, M.D., C. Kondo, A.H.T., Y. Takahashi, A.H.T., J. Maloney, B.Sc., M.Sc., Ph.D., D. Persaud, M.Sc., and S. Nicholson, M.D.
Calgary, Alberta, Canada

Digoxin-like immunoreactive substance in pregnancy 660
Antonio R. González, M.D., Stephanie Phelps, Pharm.D., Emily B. Cochran, Pharm.D., and Baha M. Sibai, M.D.
Memphis, Tennessee

Management of the diethylstilbestrol-exposed pregnant patient: A prospective study 665
Jack Ludmir, M.D., Mark B. Landon, M.D., Steven G. Gabbe, M.D., Philip Samuels, M.D., and Michael T. Mennuti, M.D.
Philadelphia, Pennsylvania

Gestational diabetes: Insulin requirements in pregnancy 669
Oded Langer, M.D., Akolisa Anyaegbunam, M.D., Lois Brustman, M.D., Denise Guidetti, M.D., and Roger Mazze, Ph.D.
Bronx, New York

Status of infants at birth and risk for adverse neonatal events and long-term sequelae: A study in low birth weight infants 676
David A. Luthy, M.D., Kirkwood K. Shy, M.D., M.P.H., Daniel Strickland, Ph.D., Judy Wilson, R.N., Forrest C. Bennett, M.D., Zane A. Brown, M.D., and Thomas J. Benedetti, M.D.
Seattle, Washington

A classification of hypertension in pregnancy based on Doppler velocimetry 680
James Ducey, M.D., Harold Schulman, M.D., George Farmakides, M.D., Burton Rochelson, M.D., Luis Bracero, M.D., Adiel Fleischer, M.D., Edwin Guzman, M.D., Denise Winter, R.N., B.S.N., and Barbara Penny, R.N.
Mineola, Stony Brook, Westchester, and Bronx, New York

(Contents continued on page 11A)

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Contents continued from page 9A

Cocaine use during pregnancy: Adverse perinatal outcome 686
Scott N. MacGregor, D.O., Louis G. Keith, M.D., Ira J. Chasnoff, M.D., Marvin A. Rosner, M.D., Gay M. Chisum, R.N., Patricia Shaw, R.N., and John P. Minogue, D.Min.
Chicago, Illinois

Neonatal morphometrics and perinatal outcome: Who is growth retarded? 691
Robert M. Patterson, M.D., and M. R. Pouliot, R.N.
San Antonio, Texas

The nonpredictive value of fetal urinary electrolytes: Preliminary report of outcomes and correlations with pathologic diagnosis 694
Isabelle A. Wilkins, M.D., Usha Chitkara, M.D., Lauren Lynch, M.D., James D. Goldberg, M.D., Karen E. Mehalek, M.D., and Richard L. Berkowitz, M.D.
New York, New York

Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short- and long-term metabolic control in gestational diabetes 699
Lois Brustman, M.D., Oded Langer, M.D., Samuel Engel, M.D., Akolisa Anyaegbunam, M.D., and Roger Mazze, Ph.D.
Bronx, New York

Early third-trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term 703
Clifford J. Bochner, M.D., Arnold L. Medearis, M.D., John Williams III, M.D., Lony Castro, M.D., Calvin J. Hobel, M.D., and Maclyn E. Wade, M.D.
Los Angeles, California

Chorionic villus sampling is associated with normal fetal growth 708
John Williams III, M.D., Arnold L. Medearis, M.D., Moraye B. Bear, M.A., and Michael M. Kaback, M.D.
Los Angeles and Torrance, California

Umbilical blood waveforms in fetal surveillance of twins 712
Luis R. Saldana, M.D., Mary Catherine Eads, R.D.M.S., and Theresa R. Schaefer, R.D.M.S.
Cincinnati, Ohio

Umbilical cord size and amniotic fluid volume in prolonged pregnancy 716
Richard K. Silver, M.D., Sharon L. Dooley, M.D., Ralph K. Tamura, M.D., and Richard Depp, M.D.
Chicago, Illinois

The origin of increased serum iron in pregnancy-induced hypertension 721
Philip Samuels, M.D., Elliott K. Main, M.D., Michael T. Mennuti, M.D., and Steven G. Gabbe, M.D.
Philadelphia, Pennsylvania

(Contents continued on page 13A)

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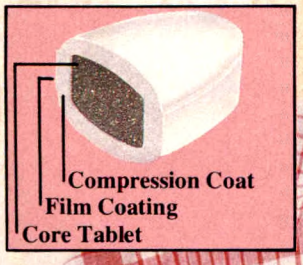
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Contents continued from page 11A

The effects of long- and short-term maternal caffeine ingestion on human fetal breathing and body movements in term gestations 726
Jack McGowan, M.D., Lawrence D. Devoe, M.D., Nancy Searle, B.A., and Roy Altman, Ph.D.
Augusta, Georgia

Leukocyte esterase activity in human amniotic fluid for the rapid detection of chorioamnionitis 730
Iffath Abbasi Hoskins, Commander, MC, USNR, Timothy R. B. Johnson, M.D., and Craig A. Winkel, Colonel, MC, USA
Bethesda and Baltimore, Maryland

Changes in cyclic adenosine monophosphate–phosphodiesterase activity in nonpregnant and pregnant human myometrium 733
Alexander D. Kofinas, M.D., James C. Rose, Ph.D., and Paul J. Meis, M.D.
Winston-Salem, North Carolina

Pregnancy outcome in medically complicated and uncomplicated patients aged 40 years or older 738
Deborah K. Lehmann, M.D., and James Chism, M.D.
New Orleans, Louisiana

Patterns of human fetal heart rate accelerations from 26 weeks to term 743
Robert Gagnon, M.D., Karen Campbell, Ph.D., Cora Hunse, R.N., and John Patrick, M.D.
London, Ontario, Canada

Riboflavin concentration in maternal and cord blood in human pregnancy 748
Nancy Wolfert Kirshenbaum, M.D., Joseph Dancis, M.D., Mortimer Levitz, Ph.D., Jean Lehanka, B.S., and Bruce K. Young, M.D.
New York, New York

The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor 753
Richard E. Besinger, M.D., Alan A. Compton, M.D., and Robert H. Hayashi, M.D.
Ann Arbor, Michigan

The significance of one abnormal glucose tolerance test value on adverse outcome in pregnancy 758
Oded Langer, M.D., Lois Brustman, M.D., Akolisa Anyaegbunam, M.D., and Roger Mazze, Ph.D.
Bronx, New York

Ultrasonic ratio of fetal thoracic to abdominal circumference: An association with fetal pulmonary hypoplasia 764
Anthony Johnson, D.O., Nancy A. Callan, M.D., Vinod K. Bhutani, M.D., Garrett H. C. Colmorgen, M.D., Stuart Weiner, M.D., and Ronald J. Bolognese, M.D.
Philadelphia, Pennsylvania

(Contents continued on page 15A)

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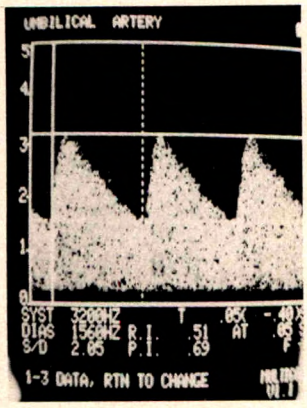


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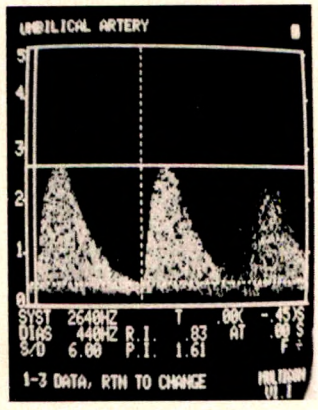
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Contents continued from page 13A

Effects of alterations of zinc-to-phosphorus ratios and meconium content on Group B *Streptococcus* growth in human amniotic fluid in vitro 770
Iffath Abbasi Hoskins, Commander, MC, USNR, Val G. Hemming, Colonel, MC, USAF, Timothy R. B. Johnson, M.D., and Craig A. Winkel, Colonel, MC, USA
Bethesda and Baltimore, Maryland

Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow 774
Kathryn L. Reed, M.D., Caroline F. Anderson, R.D.M.S., and Lewis Shenker, M.D.
Tucson, Arizona

Enhancement of maternal and neonatal natural killer cell activity with interleukin-2 780
David A. Baker, M.D., Peter O. Milch, Ph.D., William Salvatore, B.S., and Benjamin Luft, M.D.
Stony Brook, New York

CORRESPONDENCE

Maternal serum α-fetoprotein screening: Effects of weight adjustment Lillian C. Kazazian, R.N., M.S., Jennifer R. Niebyl, M.D., and J. Mehsen Joseph, Ph.D. *Baltimore, Maryland* 782

Reply James N. Macri, Ph.D., Ramana V. Kasturi, B.E., M.S., David A. Krantz, B.S., Kevin E. Koch, B.S., and Mannshya G. Hu, Ph.D. *Carle Place, New York* 783

Drug prescribing for thromboembolic disease during pregnancy Jeffrey S. Greenspoon, M.D., and Martin Montoro, M.D. *Los Angeles, California* 784

Lymphocyte-reactive antibodies and recurrent early pregnancy failure Patricia K. Biddle, M.D., Chad I. Friedman, M.D., and Peter M. Johnson, Ph.D. *Columbus, Ohio, and Liverpool, England* 785

BOOKS

Books received 787

ITEMS

Items 788

Information for authors on pages 27A, 28A, and 29A

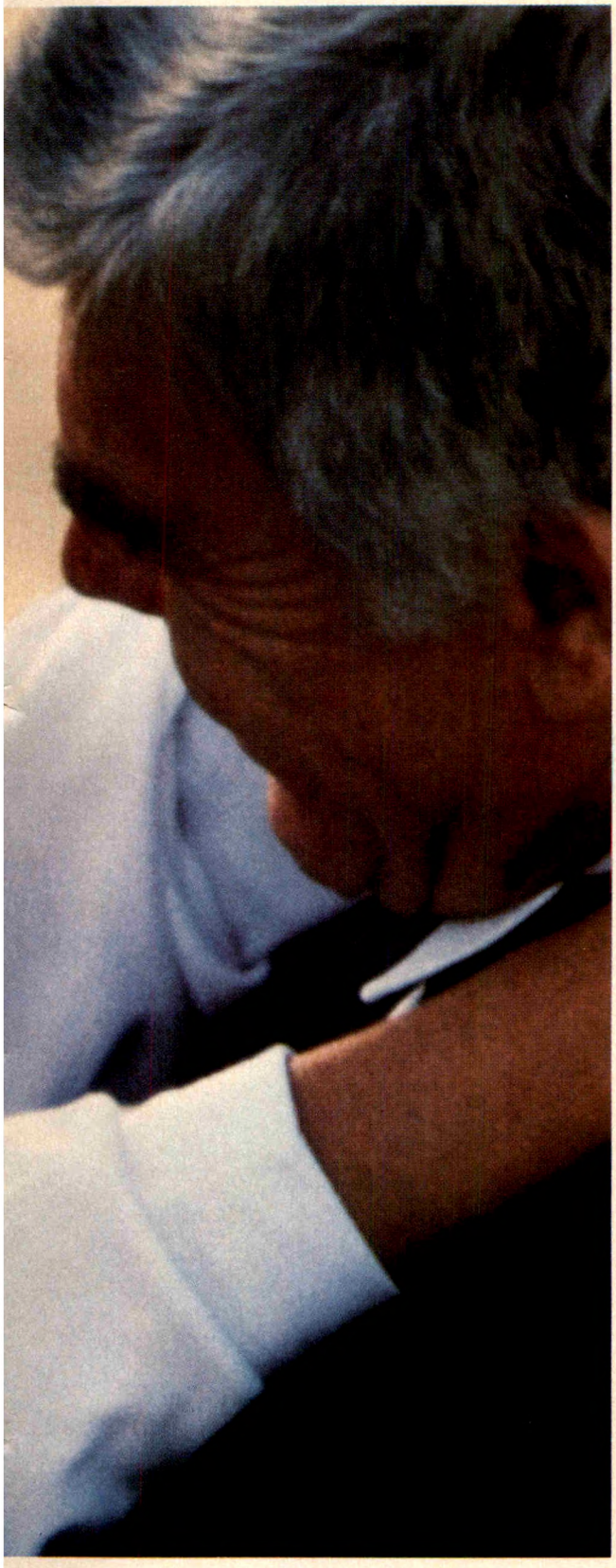
Index to advertisers on page 75A

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There's never been a better time for her



and PREMARIN®



Proven benefits beyond relief of vasomotor symptoms

No other estrogen proven effective for osteoporosis

Only conjugated estrogens tablets have established efficacy in both osteoporosis[1] and vasomotor symptoms* at 0.625 mg/day. No other estrogen, oral or transdermal, has established clinical evidence or minimum effective dose in both indications.

No estrogen proven safer

PREMARIN is the most extensively tested estrogen, with an unsurpassed record of long-term safety.
And clinical evidence shows a significantly reduced risk of endometrial hyperplasia when cycled with a progestin.[2]

PREMARIN®
(conjugated estrogens tablets)

Most trusted for more reasons

*PREMARIN is indicated for moderate-to-severe vasomotor symptoms.

Please see following page for brief summary of prescribing information.

For moderate-to-severe vasomotor symptoms and for osteoporosis

PREMARIN®
(conjugated estrogens tablets)



0.3 mg 0.625 mg 0.9 mg 1.25 mg 2.5 mg

The appearance of these tablets is a trademark of Ayerst Laboratories.

For atrophic vaginitis

PREMARIN®
(conjugated estrogens)

Vaginal Cream

0.625 mg/g



BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.
Three independent, case-controlled studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case-controlled studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semi-annual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equi-estrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing, in later life, a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30% to 90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case-controlled study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: PREMARIN (conjugated estrogens tablets, USP): Moderate-to-severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms and they should not be used to treat such conditions.) Osteoporosis (abnormally low bone mass). Atrophic vaginitis. Kraurosis vulvae. Female castration.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Concomitant Progestin Use: The lowest effective dose appropriate for the specific indication should be utilized. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of the endometrium suggest that 10 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks which may be associated with the inclusion of progestin in estrogen replacement regimens. (See PRECAUTIONS.) The choice of progestin and dosage may be important; product labeling should be reviewed to minimize possible adverse effects.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (see Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Estrogens have been reported to increase the risk of endometrial carcinoma (see Boxed Warning). However, a recent large, case-controlled study indicated no increase in risk of breast cancer in postmenopausal women. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not yet complete. If concomitant progestin therapy is used, potential risks may include adverse effects on carbohydrate and lipid metabolism.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. However, in a recent, large case-controlled study of postmenopausal women there was no increase in risk of breast cancer with use of conjugated estrogens.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.

2. *Given cyclically:* Osteoporosis. Female castration. Osteoporosis —0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off). Female castration—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (eg, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

References:

1. Lindsay R, Hart DM, Clark DM: The minimum effective dose of estrogen for prevention of postmenopausal bone loss. *Obstet Gynecol* 1984;63:759-763. **2.** Studd JWW, Thom MH, Paterson MEL, et al: The prevention and treatment of endometrial pathology in postmenopausal women receiving exogenous estrogens, in Pasetto N, Paoletti R, Ambrus JL (eds): *The Menopause and Postmenopause*. Lancaster, England, MTP Press Ltd, 1980, chap 13.

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DEFINING THE DIRECTION OF OC EVOLUTION

WITH THE ADVANCED DESIGN OF

TRI-LEVLEN™

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

No other OC regimen phases *both* hormones

▼

Provides the *lowest* monthly dose of both estrogen and progestogen

▼

Little or no effect on lipid metabolism[1-5]*

▼

Low overall incidence of breakthrough bleeding[6]

*Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These data represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to all data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

BERLEX Dedicated to innovation in OB/GYN medicine

TRI·LEVLEN™ 28
TRI·LEVLEN™ 21

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

BRIEF SUMMARY

Tri-Levlen™—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxy-gon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

References: 1. Skouby SO, Kühl C, Mølsted-Pedersen L, et al: *Am J Obstet Gynecol* 1985;153:495-500. **2.** März W, Gross W, Gahn G, et al: *Am J Obstet Gynecol* 1985;153:287-293. **3.** Vermeulen A, Thiery M: *Contraception* 1982;26:505-513. **4.** Larsson-Cohn U, Fåhraeus L, Wallentin L, et al: *Fertil Steril* 1981;35:172-179. **5.** Gaspard UJ, Buret J, Gillain D, et al: *Contraception* 1985;31:395-408. **6.** Data submitted to FDA.

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430-34

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P24,289

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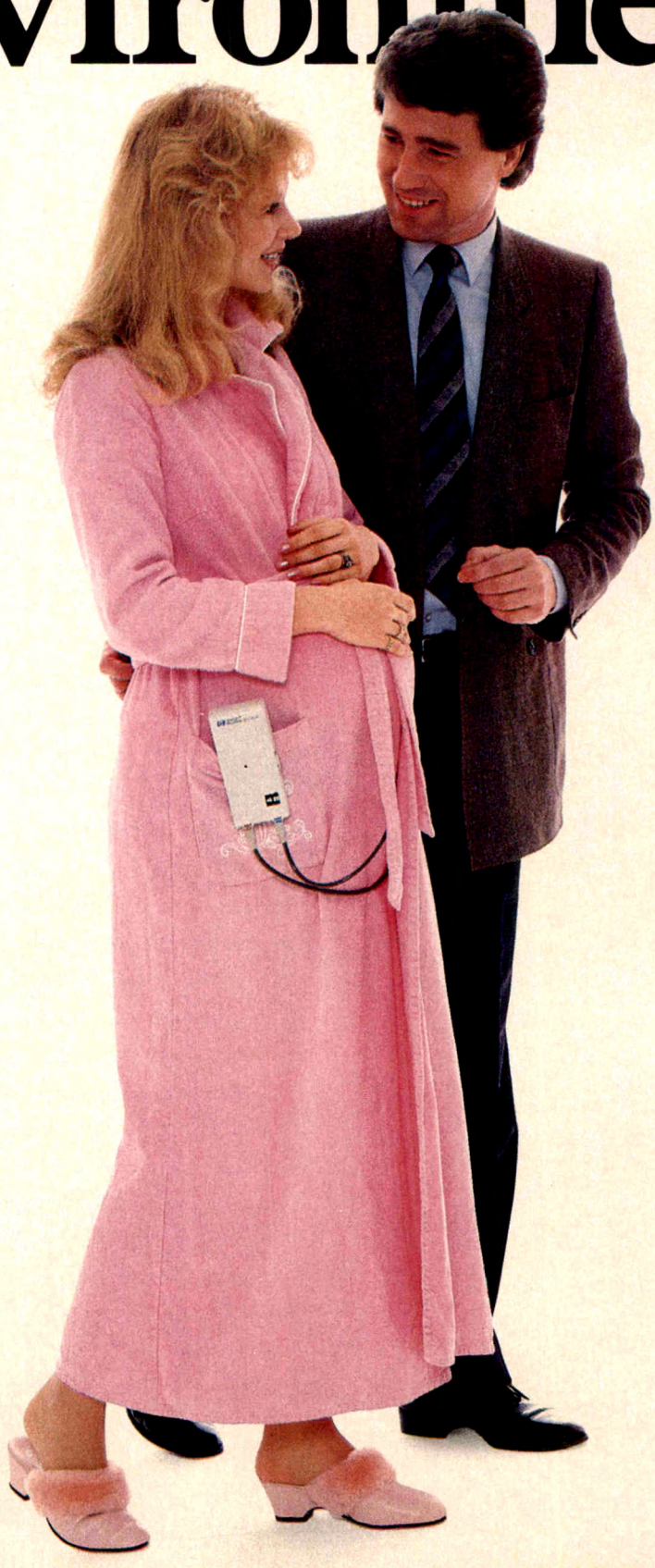
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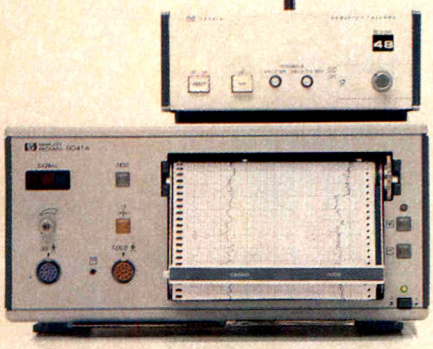
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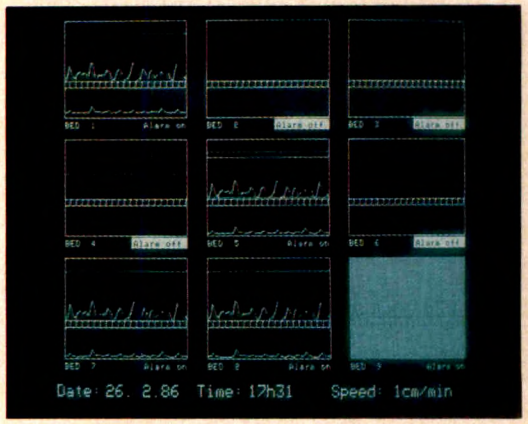
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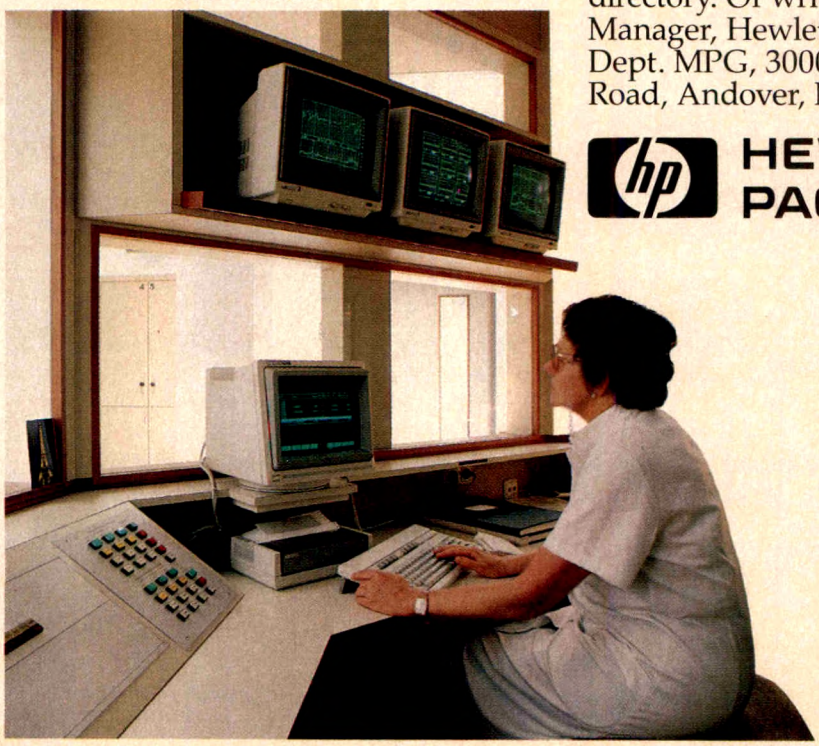
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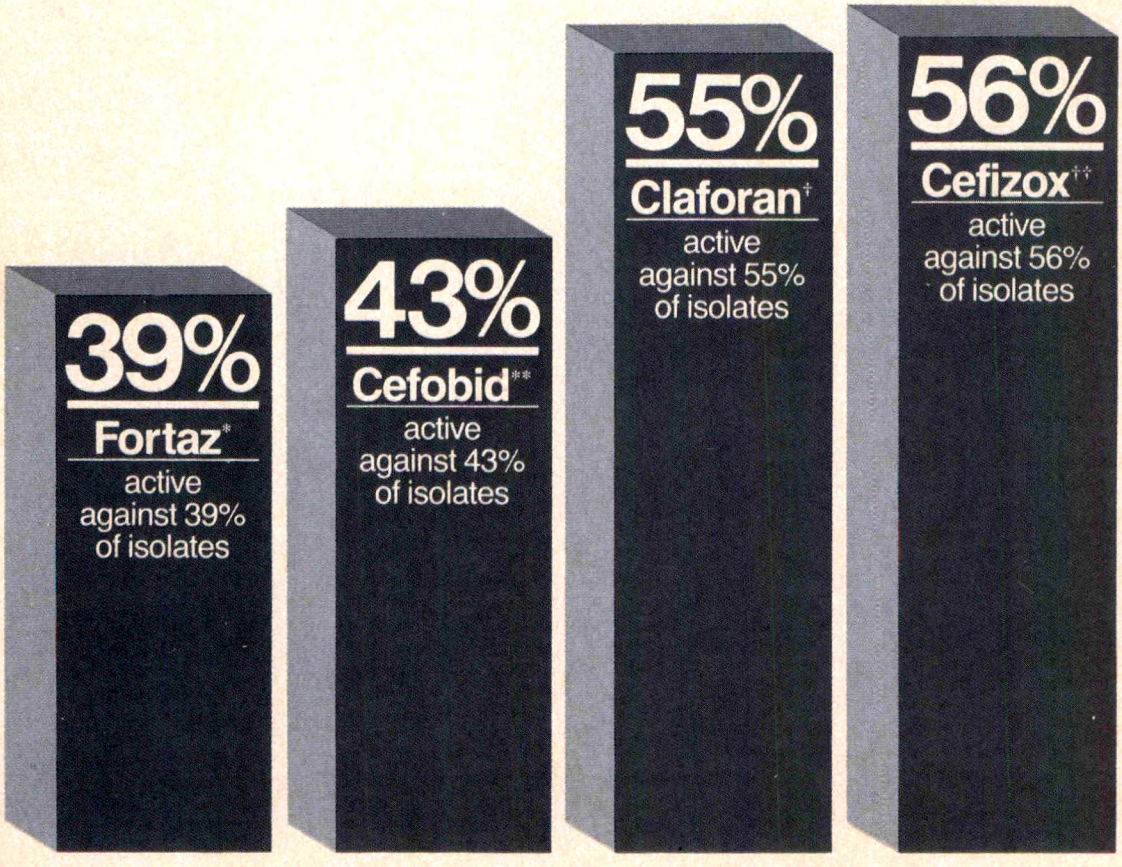
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NOTE: *In vitro* activity does not necessarily imply *in vivo* effectiveness.

MEFOXIN is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

MEFOXIN is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Streptococcus faecalis*) and many strains of *Enterobacter cloacae.* Methicillin-resistant staphylococci are almost uniformly resistant to MEFOXIN.

For a Brief Summary of Prescribing Information, please see following page.

MSD
MERCK SHARP & DOHME

Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST ‖ reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk: Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials, infusion bottles, and ADD-Vantage™# vials containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*

‖ Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

\# Registered trademark of Abbott Laboratories, Inc.

For more detailed information, consult your MSD Representative or see Prescribing Information. Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA 19486

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New from GRUNE & STRATTON, INC.

The Osteoporotic Syndrome, Second Edition
Detection, Prevention, and Treatment

Edited by
Louis Avioli, M.D.
Shoenberg Professor of Medicine, Department of Medicine
Director, Division of Bone and Mineral Metabolism
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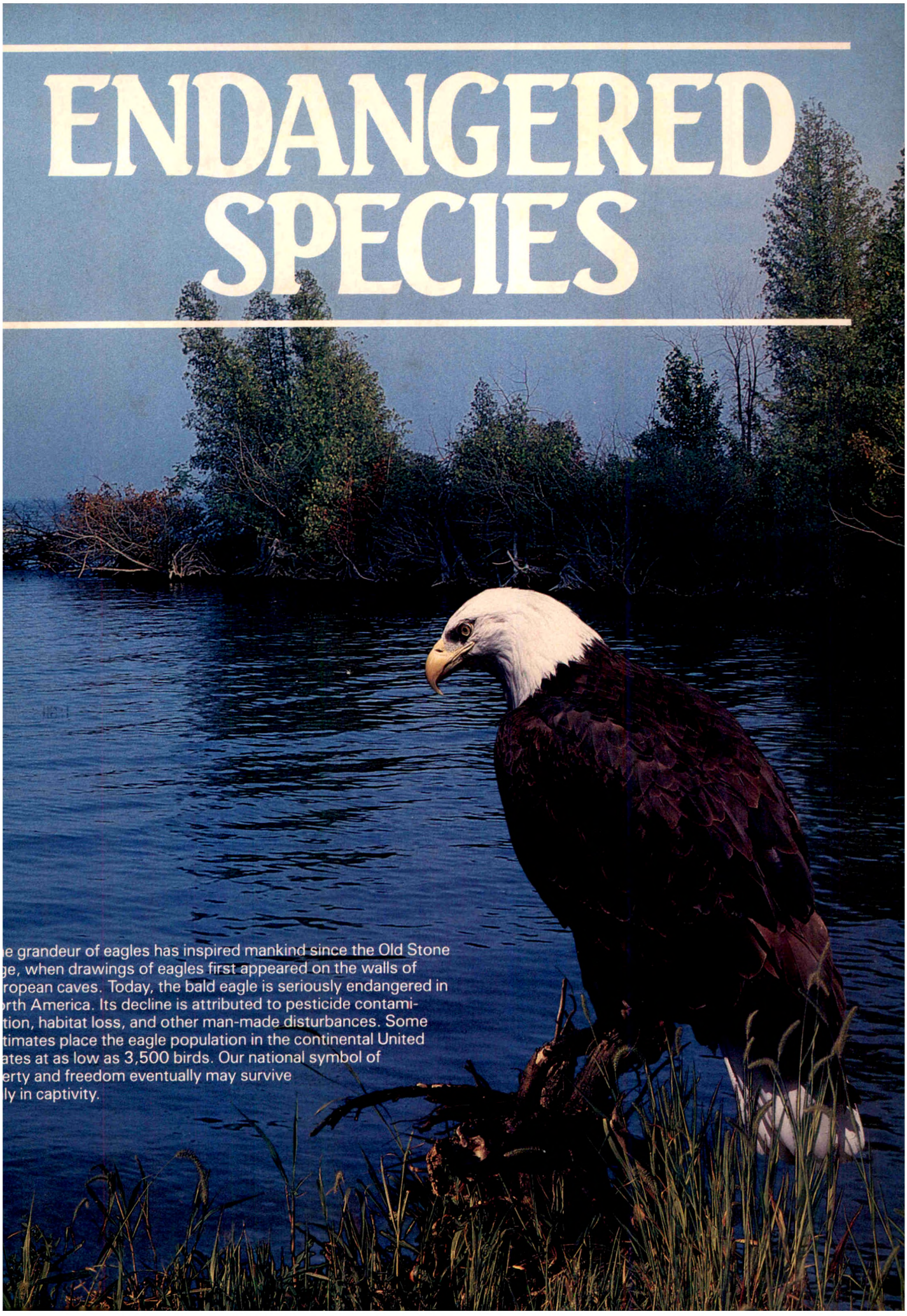
Combining new advances and information with the clear presentation of the first edition, **The Osteoporotic Syndrome, Second Edition** is a concise review of state-of-the-art techniques in the diagnosis, prevention, and therapy of the postmenopausal osteoporotic syndrome.

Like the previous edition, this text emphasizes a practical approach to the diagnosis and prevention of the osteoporotic process utilizing the most currently available knowledge. Physicians in the practice of internal medicine, orthopedic surgery, radiology, obstetrics and gynecology and rheumatology who are more apt to evaluate patients with the osteoporotic syndrome, will find this book particularly useful. Pathologists and medical or paramedical personnel in exercise physiology and nutritionists will appreciate the chapters on exercise, bone pathology, and the discussion of the role calcium plays in mitigating the potential complications of the osteoporotic problem.

Contents: Preface. Contributors. *L. Joseph Melton, III, and B. Lawrence Riggs*, Epidemiology of Age-related Fractures. *Charles H. Chesnut, III*, Noninvasive Methods of Measuring Bone Mass. *Steven L. Teitelbaum*, Osteoporosis and the Bone Biopsy. *Louis V. Avioli*, The Calcium Controversy and the RDA. *Robert P. Heaney*, Prevention of Osteoporotic Fracture in Women. *Robert Lindsay*, Estrogens in Prevention and Treatment of Osteoporosis. *Franz U. Steinberg*, Exercise in Prevention and Therapy of Osteoporosis. *Carlo Gennari and Louis V. Avioli*, Calcitonin Therapy in Osteoporosis. *Glenn Swogger, Jr.*, The Emotional Effects of the Osteoporotic Syndrome. *Linda Repa-Eschen*, Prevention and Treatment of Osteoporosis in a Healthcare Delivery System. Index.

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ENDANGERED SPECIES



e grandeur of eagles has inspired mankind since the Old Stone
ge, when drawings of eagles first appeared on the walls of
ropean caves. Today, the bald eagle is seriously endangered in
rth America. Its decline is attributed to pesticide contami-
tion, habitat loss, and other man-made disturbances. Some
timates place the eagle population in the continental United
ates at as low as 3,500 birds. Our national symbol of
erty and freedom eventually may survive
ly in captivity.

ENDANGERED SPECIES?

Is the practice of obstetrics endangered? Its survival is threatened by factors that, if allowed to continue, are likely to diminish significantly the number of practicing obstetricians. The continuing increase in the number of malpractice lawsuits against physicians and the corresponding inflation of liability insurance premiums have plunged the obstetrics specialty into a crisis.

Patient-initiated litigation is now so common that 1 in 6 obstetrician/gynecologists is sued each year, compared with 1 in 20 only ten years ago. As a result, obstetricians pay three to four times more for malpractice insurance than do physicians in most other specialties.

Survival of the practice of obstetrics is further jeopardized because fewer students are entering the specialty and the number of experienced physicians leaving obstetrics or retiring early is on the rise. For many, this crisis has taken the joy out of practicing medicine.

HARVARD SYMPOSIUM: PRESERVATION THROUGH REFORM

Immediate action is essential to preserve the practice of obstetrics. In response to this need, G.D. Searle & Co. has organized the Searle Alliance for Health Care. This initiative is helping to lead a national effort to resolve the medical professional liability crisis.

A highlight of the Searle Alliance for Health Care program is a team comprised of experts in medicine, law, insurance, and public policy. These specialists will present recommendations and position papers about reform at a professional liability symposium to be held at Harvard University on October 9–10, 1987. Concerned leaders from a variety of fields will be in attendance to hear the proceedings and recommendations as the specialists consider effective routes to medical liability reform.

Cosponsored by Harvard's Division of Health Policy Research and Education and the Searle Alliance for Health Care, the symposium will be chaired by Kenneth Ryan, M.D., Chairman of the Department of Obstetrics and Gynecology at Harvard University School of Medicine.

Beyond the Harvard symposium, the Searle Alliance for Health Care has established a national campaign to help resolve the professional liability conflict and offers many local programs to help you deal with it.

For further information on the October 9–10 Harvard symposium or to view videotaped highlights of the proceedings, contact your Searle representative.

SEARLE
Seeking resolution of the professional liability crisis

Please see the last page of this advertisement for a brief summary of the complete prescribing information.

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Demulen 1/35™

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Demulen 1/35™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients.

Demulen 1/50™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients. **Indication and usage:** For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk*: Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen. **Contraindications:** Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products. **Warnings:**

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems*: Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders*: In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction*: Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers	10.2	2.6		62.0	15.9
≥15 cigarettes daily	13.0	5.1		78.7	31.3
<15 cigarettes daily	4.7	0.9		28.6	5.7
Nonsmokers	1.8	1.2		10.7	7.4
Smokers and nonsmokers	5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases*: A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

Be alert to earliest manifestations of TE and thrombotic disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. The RCGP prospective study reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. The data suggest varicose veins have little effect on development of deep vein thrombosis but substantially increase risk of superficial vein thrombosis, risk depending on severity of varicosities. **2.** *Ocular lesions*: Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma*: Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is evidence that estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions (eg, irregular bleeding before OCs, polycystic ovaries) occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer with combination or progestogen-only OCs, though individual cases were reported. Studies show no increased breast cancer with OCs or estrogens. However, greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk of breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens rose and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other risk factors. Decrease in benign breast tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, erosion, and carcinoma in long-term users. Cervical microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increased cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. **4.** *Hepatic adenomas, etc.* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. OCs are contraindicated in case of past or present, benign or malignant liver tumors developing during use of estrogenic products. **5.** *Use in or immediately preceding pregnancy, birth defects, malignancy in offspring*: Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DES-exposed daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL for vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,000 births, compared to 7.8 for those unexposed ($P<.05$). More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. **7.** *Carbohydrate and lipid metabolism*: Decreased glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides and total phospholipids may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few months, and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but increases with longer exposure; in the 5th year it is 2½—3 times the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache*: Onset or exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating cause. **10.** *Bleeding irregularities*: Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to a higher-estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics*: In failures with combination OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. There may be increased risk of ectopics after discontinuing. **12.** *Breast feeding*: OCs may decrease quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after weaning. **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable difference in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC and troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake, unaltered free T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceride, phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask onset of climacteric. Cervical chlamydial prevalence is increased; do not assume OC protects against chlamydial PID. *Information for patients*. See patient labeling. *Drug interactions*. OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophen. **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted for: premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hemolytic uremia, malignant hypertension, itching, vaginitis, porphyria, impaired renal function, acute renal failure, sometimes irreversible, PVCs, abnormal EKG, pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythematosus, rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanoma, endometrial, cervical, and breast carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use and is available on request.**

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TRANSACTIONS OF THE FORTY-NINTH ANNUAL MEETING OF THE SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

Responsibility, obligation, and duty

Presidential address

Leo J. Dunn, M.D.

Richmond, Virginia

The opportunity to address one's colleagues in this manner on any topic that one regards important comes rarely. There are so many important matters that it is difficult to choose what thoughts one wishes to share. Having listened to presidential addresses in many organizations, I recognize that there is the opportunity to look backward on events that should not be forgotten or to stress current trends that are important to pursue or alter. Even more tempting is to predict the future. The latter I often call "highway speeches," perceiving the specialty to be at some "fork in the road" or "crossroads" where a vital decision needs to be made.

I have chosen today to speak of a current concern, one I believe that all members of this organization should share. My concern is the poor outcome of pregnancy that exists in our country and particularly that that exists in our own states. As obstetricians and gynecologists you know that the United States has a shameful record in our poor pregnancy outcome when compared with other developed countries. It is a source of national embarrassment that we are among the wealthiest of nations and yet our care of women and children, measured by perinatal, neonatal, and infant mortality rates, remains among the worst among comparable nations. We should be most concerned because the South has contributed most heavily to this poor record. Therefore I would like to present to you some data that will highlight the problem as I see it in the five states of the South Atlantic Association of Obstetricians and Gynecologists (i.e., Virginia, North Carolina, South Carolina, Georgia, and Florida). I have reviewed a great many data and have attempted to extract those data that will define the problem in a way that will point toward solutions and opportunities for our involvement. I have attempted to present the most recent complete data from our states. This has required using data for both 1983 and 1984.

I would like first to demonstrate that poor pregnancy outcome is indeed worse in our states than in the United States as a whole. Having demonstrated this, I would like to explore some of the characteristics of women who contribute heavily to poor pregnancy outcome so that we might better identify them, their unmet needs, and opportunities in which our intervention might be effective. I will attempt to prove that the most assailable common denominator of the factors that contribute to poor pregnancy outcome is unwanted pregnancies. I will particularly focus on the issue of teenage pregnancy as an important contributing cause that must be isolated for special attention. I will stress that inadequate sex education, inadequate contraceptive services, uneven access to abortion, and inadequate antepartum care are factors that contribute to the poor pregnancy results in our states. I would like to conclude with some recommendations that might be pursued by

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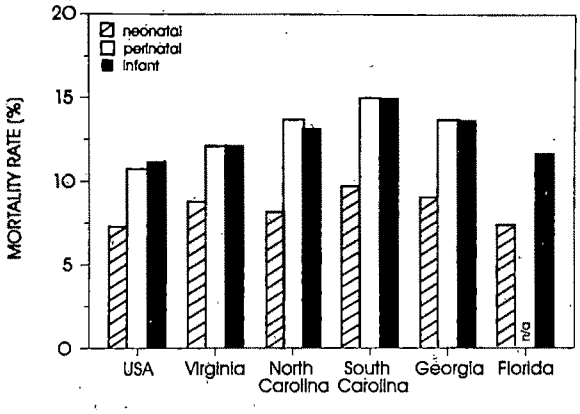


Fig. 1. Pregnancy outcome in the South Atlantic states in 1983. *Source:* RNDMU Region IV, 1986, and Virginia 1983 Vital Statistics Report. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

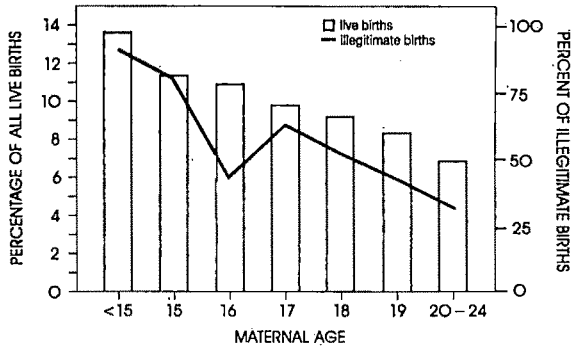


Fig. 2. Relationship between maternal age and low birth weight infants (≤2500 gm) occurring in the United States in 1984. Infants weighing ≤2500 gm account for two thirds of neonatal deaths. *Sources:* Monthly Vital Statistics Report. Hyattsville, Maryland: National Center for Health Statistics, July 18, 1986 vol 35 no. 4. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82.

all of us in our respective communities that would result in improvement of pregnancy outcome.

Fig. 1 illustrates that poor pregnancy outcome nationally is exceeded by each of our states in neonatal, perinatal, and infant mortality rates. Table I begins to offer some explanation for our poor outcome. The well-known fact that there is a marked difference between pregnancy outcome in white and nonwhite populations in this country is clearly demonstrated for the nation and for each of our states. Mortality rates in some cases are actually double for the nonwhite population. The importance of this for the South Atlantic states is the racial distribution of women of reproductive age in our states compared with the nation as a whole. It is clear that we have a much higher proportion of women at high risk for poor pregnancy outcome in our states. It is also clear to those who have toiled in this vineyard that the basic underlying factor is the relentless problem of poverty. The "war on poverty" in this country has not been won. Jones[1] of the Alan Guttmacher Institute compared the economically lowest 20% of the United States with that of other selected industrialized nations and stated that severe poverty continues in the United States whereas it has been alleviated in other nations. We as obstetricians and gynecologists can do little to reverse poverty in our states; therefore we must look for contributing factors on which we could have a positive influence.

There are certain patient characteristics that are recognized as high-risk factors in addition to race. Table II displays three of these: teenage pregnancy, incomplete education, and out-of-wedlock pregnancies. Once again, with few exceptions, our states exceed national averages for their occurrence. Exploration of these factors indicates that common among them is the issue of unwanted pregnancy.

In her tenure as President of the American College of Obstetricians and Gynecologists, a member of our society, Dr. Luella Klein,[2] was very forceful and vocal in her attempt to bring the issue of unwanted pregnancy before our specialty and the nation. Unwanted pregnancy is an enormous problem and one that is almost entirely preventable. Previous estimates have declared that >50% of the pregnancies that occur each year in the United States are unintended, many of which are unwanted.[1] In attempting to determine a rough estimate of the unwanted pregnancies in our states, I have used an artificial calculation you may criticize. However, I believe it gives a conservative estimate of unwanted pregnancies in our states. My calculation requires four basic assumptions. The first is that all pregnancies of married women are wanted. We know this to be untrue and the extent to which it is untrue makes the final calculation a conservative one. The second assumption is that all out-of-wedlock pregnancies are unwanted. This also has an error, but even in today's changed morality the probability of error is likely <10%, even for adults. The third assumption is that women who undergo voluntary induced abortion have unwanted pregnancies. The probability of error in this assumption seems small. Fourth is the assumption that spontaneous abortions occur with equal frequency whether the pregnancy is wanted or unwanted.

Based on these assumptions, my calculations in Table III indicate that between 40% and 47% of all pregnancies that occur in our states can be classified not only as unintended but as unwanted. If true, this indeed identifies an enormous problem that has serious health, social, economic, and emotional implications that affect more than a quarter of a million women each year in

Table I. South Atlantic states: pregnancy outcome, 1983

	Neonatal mortality rate (%)		*Perinatal mortality rate (%)*		*Infant mortality rate (%)*		*Racial distribution in women 15-44 yr (%)*	
	W	*NW*	*W*	*NW*	*W*	*NW*	*W*	*NW*
United States	6.4	10.8	10.8	15.1	9.7	16.8	85	15
Virginia	7.1	13.5	10.2	17.8	9.6	19.7	78	22
North Carolina	7.0	12.5	11.2	19.0	10.5	19.1	74	26
South Carolina	7.0	14.0	11.1	21.0	11.2	20.7	67	33
Georgia	6.9	13.2	10.5	19.6	9.9	20.4	70	30
Florida	6.4	11.1	n/a	n/a	9.9	18.6	80	20

W, white; NW, nonwhite.

Source: RNDMU Region IV, 1986, and Virginia 1983 Vital Statistics Report. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

Table II. South Atlantic states: high-risk factors, 1983 to 1984

	% Total births women aged 17 yr or less			*% Total births women with education < grade 12*			*% Total births women unmarried*		
	T	*W*	*NW*	*T*	*W*	*NW*	*T*	*W*	*NW*
United States	4.8	3.7	9.1	20.9	18.0	32.1	21.0	13.4	59.2
Virginia	4.5	3.1	8.4	20.6	16.8	31.8	20.8	9.9	55.8
North Carolina	6.3	4.4	10.7	24.9	21.5	32.4	21.2	7.9	53.4
South Carolina	6.8	4.2	10.8	26.0	21.8	32.4	25.6	8.3	53.0
Georgia	7.1	4.8	11.4	26.8	23.3	33.2	25.1	8.3	56.8
Florida	5.6	3.7	11.3	25.0	20.8	37.2	25.1	13.0	63.1

T, total all races; W, white; NW, nonwhite.

Source: RNDMU Region IV, 1986, and Virginia 1983 and 1984 Vital Statistics Reports. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

Table III. South Atlantic states: calculation of unwanted pregnancies

	Out-of-wedlock births (n)	*Induced abortions (n)*	*Live births plus induced abortions (n)*	*% of unwanted pregnancies*
United States	770,355	1,268,987	4,938,128	41.3
Virginia	17,195	31,339	114,058	42.3
North Carolina	18,205	32,952	118,994	40.0
South Carolina	12,967	12,164	62,834	40.0
Georgia	23,076	34,514	126,527	45.6
Florida	38,966	64,444	219,841	47.0

Sources: Monthly Vital Statistics Report. Hyattsville, Maryland: National Center for Health Statistics, July 16, 1986 vol 35 no. 4. Abortion Statistics, US 1982-83. Atlanta, Georgia: Centers for Disease Control, 1986.

our combined states. It most certainly must affect the individuals born of these pregnancies for a lifetime. Although unwanted pregnancy is an issue for all races and all ages of women, Tables IV and V demonstrate the particular preponderance of this problem for black women and adolescents.

No national or regional statistics characterize pregnancies in their outcome by whether they are wanted or unwanted. Therefore, to demonstrate that unwanted pregnancy is indeed related to poor pregnancy outcome, I have looked at outcome that is related to those factors that I believe identify them. We must look at pregnancy outcome in the very young in whom out-of-wedlock pregnancy rates are very high and in whom abortions are very frequent. We must also look at the pregnancy outcome in nonwhite women as compared with white women. As McCormick[3] has emphasized, the most important determinant of neonatal, perinatal, and infant deaths is that of low birth weight. Therefore I have chosen this as my indicator because it will include both morbidity and mortality rates. Births of infants of low weight are, in fact, the most important challenge in current obstetrics because its prevention must be achieved if pregnancy outcome is to be improved. In

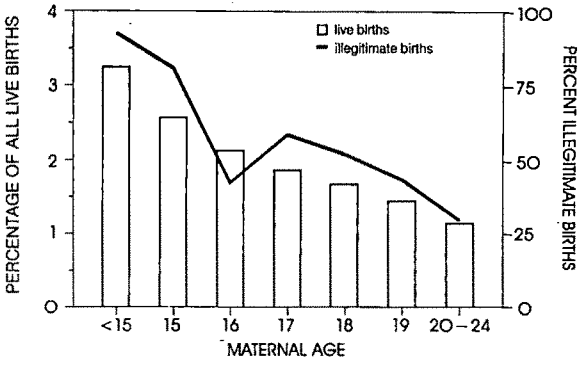


Fig. 3. Relationship between maternal age and very low birth weight infants (≤1500 gm) occurring in the United States in 1984. Infants weighing ≤1500 gm account for one half of neonatal deaths. *Sources:* Monthly Vital Statistics Report. Hyattsville, Maryland: National Center for Health Statistics, July 18, 1986 vol 35 no 4. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82.

Table IV. Out-of-wedlock births by age and race of mother in the United States, 1984

Maternal age (yr)	*% Mothers unmarried*		
	White	*Black*	*All races*
<15	80.7	98.5	91.1
15	70.2	97.6	82.6
16	59.2	96.1	72.9
17	49.8	93.2	63.4
18	40.2	88.5	53.9
19	31.0	81.6	43.7
20-24	15.8	62.7	24.5

Source: Monthly Vital Statistics Report. Hyattsville, Maryland: National Center for Health Statistics, July 16, 1986 vol 35 no. 4.

Figs. 2 and 3, using national data, I have demonstrated the relationship between maternal age and the occurrence of both low birth weight and very low birth weight infants. I have superimposed the incidence of out-of-wedlock pregnancy over this age range. The relationships here seem very clear. In Fig. 4 I have simply recorded the occurrence of live births of low birth weight, separated by race, in each of our states and compared them with national figures. Once again is demonstrated the influence of race and the poor outcome in our states compared with that of the whole country. I believe these figures support the claim that those factors identified as defining unwanted pregnancy are related to both low birth weight and poor pregnancy outcome. Although it is not the entire solution to the problem I would like to predict that a strong and concerted effort in our states to prevent unwanted pregnancy, if successful, would have a profound effect on pregnancy outcome within a relatively short period of time.

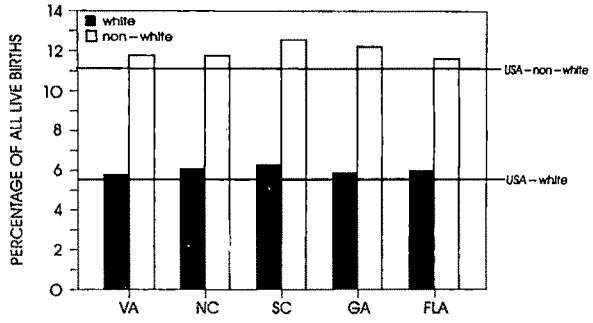


Fig. 4. Relationship between race and low birth weight infants (≤2500 gm) in South Atlantic states as compared with the United States in 1984. *Sources:* RNDMU Region IV, 1986, and Virginia 1984 Vital Statistics Report. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

Table V. Legal abortions: ratio to 1000 live births by maternal age

Maternal age (yr)	*No. of abortions*	*Ratio: 1000 live births*
<15	9,693	1,337
15-19	282,437	722
20-24	378,711	407
25-29	224,233	247
30-34	115,307	237
35-39	51,446	379
>40	16,067	777
Total	1,094,775	364

Source: Abortion Statistics, US 1982-83. Atlanta, Georgia: Centers for Disease Control, 1986.

I stated initially that I wanted to focus specifically on the issue of teenage pregnancy. Although teenage pregnancy does not represent the majority of unwanted pregnancies, the high percentage of unwanted pregnancies and the relatively tragic aspect of teenage pregnancy demands that we pay special attention to this issue. Prevention of teenage pregnancy is clearly a major public health issue, not only for our region but for the United States as a whole. Although it is neither a realistic nor necessarily desirable goal, the prevention of pregnancy in women 19 years of age or younger would reduce births of low weight in this country by 18% and by a higher percentage in our region. There are a number of myths in regard to teenage pregnancy that are important to dispel. There is a common belief that these women and disadvantaged women of all ages deliberately undergo pregnancy because of financial rewards of public assistance programs, etc. The study of Jones[1] from the Alan Guttmacher Institute in 1985 showed clearly that such financial advantages are fewer

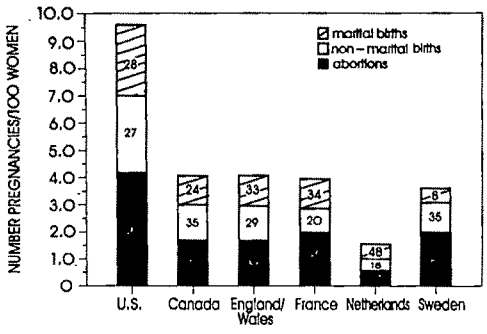


Fig. 5. Comparison of United States with other developed nations in regard to pregnancy rates, marital status, and abortion for women aged 15 to 19 years in 1980 and 1981. (Reprinted with permission from Jones EF, Forrest JD, Goldman N, et al. Teenage pregnancy in developed countries: determinants and policy implications. Fam Plann Perspect 1985; 17:53.)



Fig. 6. Prevalence of sexual activity by age in the United States in 1986 for boys and girls aged 12 to 17 years. *Source:* Taylor H, Kagay M, Leichenko S. American teens speak: sex, myths, T.V., and birth control. The Planned Parenthood Poll, Project No 864012. Louis Harris and Associates, Inc., October 1986.

in the United States when compared with those of other nations of comparable economic level and with fewer teenage pregnancies.[1] It is also commonly stated that many of these children become pregnant because they have no ambitions for personal success or see no such opportunities. A recent Planned Parenthood survey dispels that myth by demonstrating that 94% of all teenagers have high personal aspirations that clearly will be thwarted by unwanted pregnancy.[4] It is important to recognize the severe damage that unwanted pregnancy does to the lives of these young individuals. Half of these women will never complete their education and many will be forced to live the rest of their lives in poverty because early childbearing has limited their opportunities.[5] This entrapment of our youth by unwanted pregnancy is indeed an American tragedy that contributes to many societal ills such as poverty, welfare dependence, and child abuse.

Fig. 5 taken from the Jones study[1] dramatically demonstrated that the United States has a dismal record of teenage pregnancy when compared with other industrialized modern nations. Notice that there are as many or more abortions among teenagers in our country than there are pregnancies in teenagers of nations with whom we were compared. Jones also demonstrated that this is not simply a racial issue because the pregnancy rate among white American teenagers alone exceeded that of teenagers in all the other nations with which they were compared. It is not true that teenagers in our country are more sexually active than those in other nations because their study showed that our adolescents are at the mean for all of these countries in sexual activity. The important difference is clearly in the prevention of pregnancy. Unwanted pregnancy is a sexually transmitted disease and prevention must be achieved.

Prevention of teenage pregnancy has been approached in other nations by strong educational programs linked with contraceptive services and abortion available for management of the failures. This was achieved in these other nations by the public consensus of a need to solve a critical national problem. In our country, because of the influence of diverse groups who become involved in public debate, we are chronically unable to develop a public policy or an organized approach to solve our problem. Therefore a national solution to this problem never will be forthcoming, in my opinion. It is of vital importance that state and local solutions be developed and it is in this arena that we can be effective.

Analysis of public school sex education programs in metropolitan areas has indicated that only 34% of these are rated as comprehensive.[6] Dr. Koop and Secretary Bennett will need to present their thoughts more clearly if I am to be convinced that sex education in our schools is important and appropriate to prevent acquired immunodeficiency syndrome but not to prevent pregnancy. This dilemma was clearly demonstrated in our community in the past month in which the reports of the Planned Parenthood Survey and Dr. Koop's recommendations were countered by editorials expressing skepticism about sex education in the schools.[7, 7a] Little progress has been made in spite of the fact that the majority of the public support such education. Public debate in this country is not focused on the problem but only on the solutions. Very vocal groups insist that solutions be limited to the prevention of premarital sex and no other solution is acceptable. I agree that teaching children not to play with matches is appropriate. However, when the house is on fire a lecture on playing with matches is neither effective nor appropriate. This house is on fire.

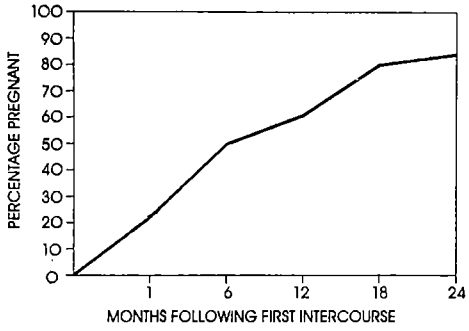


Fig. 7. The relationship between the time interval between first intercourse and conception in women aged 18 and 19 years with out-of-wedlock pregnancies. (Reprinted with permission from Teenage pregnancy: the problem that hasn't gone away. New York: The Alan Guttmacher Institute, 1981.)

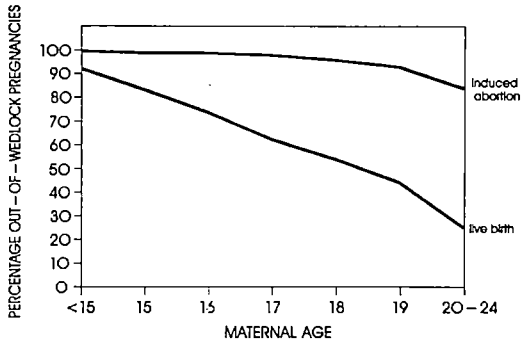


Fig. 8. The relationship of age on the out-of-wedlock status of women who undergo abortion versus live birth in Virginia in 1983. *Source:* Virginia 1983 Vital Statistics Report. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

Good comprehensive sex education begun early enough is not likely to increase teenage sexual activity but will help in preventing unwanted pregnancy.[8] Because our school system has repeatedly failed to accomplish this, perhaps we as obstetricians and gynecologists can be helpful in this arena in our communities. It has been stated that teenagers in the United States are learning sexual behavior by seeing >9000 sexual events on television each year. None of these events address the issues of responsibility or contraception. At present both the American College of Obstetricians and Gynecologists and Planned Parenthood are trying to reverse existing network policies against contraceptive information. Planned Parenthood is currently asking for our support in appealing to the networks to change this policy and I would advocate that each of us support this effort vocally and financially. Consumers can also support this effort very effectively if they would be informed as to the sponsors of the programs that promote unbalanced information. My second recommendation in regard to education is that the American College of Obstetricians and Gynecologists be petitioned to develop more sex education material that can be used by us, other health professionals, educators, parents, or the individual himself or herself. The American College of Obstetricians and Gynecologists recently produced an excellent educational tape on physical exercise for home use to improve women's health. Could they not produce similar material on sex education using a medium attractive to our teenagers? Could they develop seminars for those willing and qualified to be educators in our communities? I believe that this would be an important educational endeavor and a method by which the misinformation held by the public in regard to contraception such as the fear of carcinogenesis could be dispelled and corrected.[10] Working in concert with national, regional, and local organizations that are already pursuing these goals, you and I could play an important role in improving sex education, especially for this vulnerable group. Such educational programs can be devised to improve self-esteem, alter what is considered normative behavior, establish a sense of responsibility, and provide the knowledge necessary to avoid pregnancy and disease.

The second area of my concern is in regard to contraceptive services. It has been shown that if sex education is not linked to the availability of contraceptive services it is far less effective. The nature of the problem can be better defined by first showing the degree of sexual activity of our teenagers at the present time. Fig. 6 shows the latest available figures from a 1986 Planned Parenthood poll conducted by Louis Harris and Associates.[4] You can see from these figures that involvement in sex begins at a very early age with 7% of girls being sexually active at the age of 12 years. Before the age of 17 years more than half of both boys and girls have engaged in sexual intercourse. Fig. 7 indicates the interval between first intercourse and conception in 18 and 19 year olds.[11] Considering adolescents of all ages, Dryfoos[12] has stated that one third become pregnant within the first 24 months regardless of the age of onset of intercourse. Surveys have demonstrated that on the average a teenager will be sexually active for >16 months before she seeks help with contraception from available clinics.[13] Therefore, recognizing the early age of sexual activity and long delay before seeking contraceptive services, it is evident that to be effective sex education must be early and linked to provision of contraceptive services. The most effective way to accomplish this is to provide such clinics in the public schools. Your voice in your local community school board meetings presenting the information I have outlined today, with your recommendation that this is an important solution to the problem, would be

Table VI. Live births and induced abortions by age in Virginia, 1983

	Maternal age (yr)						
	<15	*15*	*16*	*17*	*18*	*19*	*20-24*
Live births	234	542	1123	1900	2862	4073	25,097
Induced abortions	354	668	1204	1706	2352	2448	10,349
Ratio/1000 live births	1512	1232	1072	898	824	601	412

Source: Virginia 1983 Vital Statistics Report. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

very influential. If the obstetric and gynecologic society in your community, as a body, addressed the school board with such recommendations and the willingness to voluntarity participate as teachers and providers, it could hardly be ignored. I know of no instance in which an obstetric-gynecologic society has made such a direct request to any school board.

Teenagers have specific needs for contraceptive services that are not being met. Teenagers require direction toward these services; their self-motivation is not sufficient. They are neither knowledgeable nor adept at accessing health care for themselves. Impediments must be recognized and removed. The services must be readily available and this is why I advocate school clinics as the ideal for the 75% of teenagers who are enrolled in school. Community contraceptive clinics that are open only while teenagers are in school are inaccessible. For the other 25% and for those communities in which the schools will continue to reject recommendation for such clinics, neighborhood clinics or physician's offices would be the alternatives. It is very important that these services be confidential wherever they are provided. Parental consent requirements interfere with women's willingness to seek help and the physician's willingness to prescribe.[14] Recognize that as parents you do not discuss your sexual practices with either your parents or your children. Expecting otherwise from teenagers is unrealistic. State laws authorizing minors to give their own consent increase both patient and physician participation.

Services provided must be inexpensive or free. The usual and customary rates for office visits and oral contraceptives are out of the financial reach of most of these young people. State budgets for contraception rely too heavily on federal sources and too little on state resources to meet the needs.[15] Oral contraceptives should be made available by the state at low cost to teenagers whether the individual receives her prescription from a clinic or a private physician. The requirement for pelvic examination before the prescription of oral contraceptives is stated to be a deterrent by 69% of teenagers.[4] Although it will be controversial, I believe it is important for our specialty to further evaluate the risk versus benefit of pelvic examination for the initiation of oral contraception for the young teenage woman. Elimination of this requirement would increase the use of effective oral contraception by the young teenager, and the risks versus benefits of this approach should be seriously reconsidered.

Therefore, we as obstetricians and gynecologists can be effective in providing contraceptive education and contraceptive services by our individual efforts, through our societies, through local school boards, in concert with Planned Parenthood, and through public health clinics, by providing services on a voluntary basis. Although many nonmedical civic-minded individuals are making efforts toward the prevention of pregnancy, only you and I are legally able to prescribe the most effective forms of contraception, and therefore we have a responsibility to participate.

Even under ideal circumstances contraceptive failures occur. In her Presidental Address to the American College of Obstetricians and Gynecologists, Dr. Klein[2] emphasized this fact and the role of abortion for these unwanted pregnancies. Induced abortion has been determined as a legal right of women in this country but remains a controversial and divisive issue. I have already demonstrated the relatively higher use of abortion in teenagers as compared with other age groups. This is further demonstrated in Table VI giving information from the state of Virginia on the relative use of induced abortion by age groups for 1983. One might imply from these figures that abortion is readily available to these individuals. However, Fig. 8 demonstrates the proportion of out-of-wedlock pregnancies by outcome and age group for the same state during the same period. This information casts a different light on the issue. One can see that the decision-making process for continuation of an out-of-wedlock pregnancy or the use of induced abortion varies markedly by the individual's age. Freedom of choice and accessibility of services may indeed be related to the individual's ability to act with independence. The Supreme Court ruled that access to induced abortion is a woman's right. Subsequent actions by federal and state legislators have denied access to induced abortion for the underprivileged by the economic maneuver of denying public funds for these procedures. Therefore, in the United States today the individual who has the least access to abortion services is the very young, black teenager with an out-of-wed-

Table VII. South Atlantic states: out-of-hospital deliveries, 1984

	All births	*By midwife*		*Out of hospital*	
		No.	*%*	*No.*	*%*
Virginia	79,365	902	1.1	585	0.7
North Carolina	86,626	1443	1.7	427	0.5
South Carolina	48,296	1275	2.6	447	0.9
Georgia	93,770	3670	3.9	304	0.3
Florida	155,199	9835	6.3	1452	0.9

Table VIII. South Atlantic states: ratio of physicians to annual live births

	*Births (No.)**	*ACOG fellows (No.)†*	*Deliveries/fellow (No.)*	*AMA M.D. OB-Gyn (No.)‡*	*Deliveries/OB-Gyn (No.)*
Virginia§	82,719	536	154	678	122
North Carolina	85,986	556	155	665	129
South Carolina	50,561	278	182	311	163
Georgia	92,258	572	161	695	133
Florida	155,344	969	160	1285	121

**Source:* RNDMU Region IV Network. Washington DC: Department of Health and Human Services, 1986.

†*Source:* American College of Obstetricians and Gynecologists Fellowship Statistics, 1986.

‡*Source:* American Medical Association Physician Characteristics and Distribution in the U.S., 1984.

§*Source:* Virginia 1984 Vital Statistics Report. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

Table IX. South Atlantic states: ratio of obstetric/gynecologic residents to out-of-wedlock births, 1984

	*Out-of-wedlock births (No.)**	*Residents in OB-Gyn programs (4 yr)†*	*Deliveries resident/yr (No.)*
Virginia‡	17,195	104	165
North Carolina	18,205	96	190
South Carolina	12,967	40	324
Georgia	23,076	96	240
Florida	38,966	125	312

**Source:* RNDMU Region IV Network. Washington DC: Department of Health and Human Services, 1986.

†*Source:* ACGME Directory of Residency Training Programs, 1986-1987.

‡*Source:* Virginia 1984 Vital Statistics Report. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

lock pregnancy. By some distortion of thinking this is considered constitutionally correct and in keeping with democratic principles.

Most of you belong to medical organizations that have lobbying services at state and federal levels and to which you contribute regularly. One of the strongest lobbies in the United States today is that of the medical profession. If they are to have credibility, these lobbies must be used not only for the good of the profession but also for the public good. If you have the conviction that we must solve the issue of unwanted pregnancy to improve the outcome of pregnancy, you must make provision of contraception and the provision of abortion services an issue in your state. Through these lobbying mechanisms we can insist on the legislative changes that would make these services accessible to the needy but disenfranchised. Using our lobbying efforts only for purposes of our own self-interest weakens our credibility when we turn to the public for their support for issues such as the current malpractice controversy. Perhaps the lack of public concern about our malpractice dilemma is a reflection of their perception of our lack of concern for the public in issues such as the one I am bringing before you today. If we are clearly an advocate of the public's welfare I believe they will more clearly be an advocate of ours.

I would like to turn away from these comments regarding prevention of pregnancy to address those pregnancies that do continue to delivery. It is clear that socially and economically disadvantaged pregnant women have a worsened pregnancy outcome. Several studies including those of Peoples and Siegel[16] and Showstack et al.[17] have demonstrated that inadequate antenatal care is an important contributing factor to the birth of low birth weight infants. Table VII shows that in our states we have made important progress in providing hospital delivery services for almost all women. It also demonstrates that almost all births are attended by physicians. Our continued weakness is in the provision of antenatal care. Fig. 9 indicates that nationally and locally we have an unacceptable number of women who receive no prenatal care or late prenatal

care. If the American College of Obstetricians and Gynecologists' standards of number of prenatal visits were applied, the percentage of inadequate antenatal care is even higher. Surely the provision of obstetric care must be regarded as a key responsibility of the obstetrician/gynecologist.

We do not have a severe manpower shortage in obstetrics and gynecology in the South Atlantic states. Table VIII demonstrates the ratio of obstetric practitioners to live births in our states giving both those listed by the American College of Obstetricians and Gynecologists as fellows and those listed by the American Medical Association as doing any obstetric practice. The figures of the American College of Obstetricians and Gynecologists also include some residents-in-training who are registered as junior fellows. Table IX addresses the issue of whether there is a need for practitioners versus residents to participate in the care of medically indigent patients. There seems to be a growing concept that such patients should be relegated to hospitals that have residency training programs "for the good of the residents." Because data are not available on medically indigent obstetric patients, I have taken as an assumption that the out-of-wedlock pregnancies in our states would constitute the majority of those patients who are medically indigent. This is at best an approximation but demonstrates that it is not reasonable to expect our residency training programs to assume responsibility for the entire indigent obstetric population. Therefore, it continues to be necessary that the practitioner of obstetrics and gynecology participate in their care.

Many of us willingly and voluntarily participate in the provision of health services to the medically indigent. When I arrived in Virginia 20 years ago a Public Health survey indicated that the average obstetrician/gynecologist spent 17% of his or her time providing services to the poor. Indeed that has continued in many of our communities in which physicians I know have given up to as much as one third of their time in the provision of services to the community's needy. I agree that this voluntary provision of services has not been given sufficient public recognition, and our practitioners have received little or no credit for these services in the past. My concern is that this volunteerism and sense of responsibility is becoming altered, distorted, and now avoided in many communities. Newspapers report increasing incidents of community physicians and community hospitals refusing care to indigent obstetric patients. In prior years in our community, many of the leading obstetricians and gynecologists were seen regularly in our public clinics and inpatient services for purposes of teaching and giving service. Today in our community, with few notable exceptions, the leading obstetricians and gynecologists in our community are never seen giving such public service. Both proprietary and community hospitals ma-

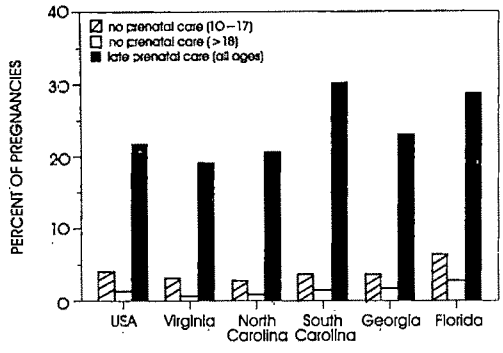


Fig. 9. Comparison of inadequate prenatal care in the South Atlantic states with that occurring in the United States in 1984. *Source:* RNDMU Region IV, 1986, and Virginia 1984 Vital Statistics Report. Richmond, Virginia: Center for Health Statistics, Virginia Department of Health.

nipulate in every way possible to avoid providing care to these medically needy patients. The stated reason for this change among our practitioners and their hospitals is the malpractice climate. It is alleged that the indigent population is more litigious than other segments of our society. It is declared manifestly unfair both to provide free care and to be at risk for litigation for doing so. Although I do not question the unfairness of this situation, I do wish to express doubt that this group of individuals are indeed more litigious than any other segment of our population. I feel that this issue should be studied and predict that the outcome will prove that they are not. However, the unfairness issue is valid and needs to be addressed. My recommendation is that obstetricians and gynecologists, through their appropriate medical societies, recommend legislation in their states to apply "good samaritan" rules to physicians who provide free medical services to the needy and make it an obligation of the state to indemnify physicians for these charitable acts. Such legislation is under serious consideration for submission to the state legislation in Virginia this year. If these two things can be accomplished, I would hope with that reassurance that every practitioner of obstetrics and gynecology would accept as a personal responsibility the contribution of free service to the medically needy obstetric patient. This has been a tradition in our specialty that is becoming distorted in an unattractive way by both malpractice and commercial competition that are reshaping our profession in ways that we must resist.

What I have attempted to do in this short time is to bring to your attention as obstetricians and gynecologists that there continues to be a significant problem of quality of reproductive outcome in the states in which we live and practice. I have tried to put forth the argument that a basic cause of this poor outcome derives from the high numbers of unwanted pregnancies that occur in our states. Reduction in the rates of these

unwanted pregnancies requires our intervention at various levels.[11] I must justify the reason for bringing these matters and these recommendations to the attention of the South Atlantic Association of Obstetricians and Gynecologists. My belief is that this society is composed of individuals invited into its membership on the basis of being an outstanding obstetrician/gynecologist in his or her state. This is a society that presumably represents the elite practitioners, teachers, and researchers of obstetrics and gynecology in our five states. If I have represented to you correctly that the problem of the outcome of pregnancy in our states is the most important health problem related to women under our care, I believe it is an issue important for this society to discuss and determine in what ways it can affect change. I must express the opinion that we have not provided leadership in the effort to solve this critical problem. The way has already been paved by such organizations as Planned Parenthood, the Guttmacher Institute, the March of Dimes, the Southern Governors Regional Task Force on Infant Mortality, Healthy Mothers, Healthy Babies Coalition, and others. Recent efforts on the part of Nurses Association of the American College of Obstetricians and Gynecologists and American College of Obstetricians and Gynecologists such as the establishment of standing committees on underserved women and adolescent care will direct our national involvement, but you and I must attempt to solve the problem at the local level. For example, the concern by the governors of our states has led to new provisions in the federal Medicaid Law that would specifically allow pregnancy care for the many women who now have no support. Concerned groups in our states who recognize the value of antepartum care are lobbying actively in behalf of these women to urge the state legislators to adopt the program. Few obstetricians are even aware of this important legislation and state obstetric/gynecologic societies in the South Atlantic states are too engrossed in issues of tort reform to lobby in favor of it.

In my view effectiveness in bringing about change will require a coordination of efforts of our various professional societies together with the efforts of those organizations that have expressed concern and already made efforts to improve pregnancy outcome. The South Atlantic Association is a society of considerable prestige and influence in our five states. It is composed of you outstanding men and women to whom society has given the right to practice obstetrics and gynecology in a setting of your choice with substantial economic reward. John D. Rockefeller stated in 1941 "I believe that every right implies a responsibility; every opportunity, an obligation; every possession a duty." I can only express my hope to you that the President of the South Atlantic Association at the end of this century will choose for his or her address to look backward and will be able to report with pride our achievements in improved outcome of pregnancy.

I appreciate Rae Grad of the Southern Governors' Association, Judy Fielden of the American College of Obstetricians and Gynecologists, Beverly Derr of the Statistics Bureau of the Virginia State Health Department, and Cathy McCray of the Medical College of Virginia for their assistance in obtaining the data presented here.

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A prospective comparative study of the extended nonstress test and the nipple stimulation contraction stress test

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The nonstress test and the nipple stimulation contraction stress test were performed at the Medical College of Georgia and the University of Mississippi Medical Center, with the use of common maternal state protocols, minimum criteria for baseline fetal heart rate reactivity, and follow-up of abnormal test results. At the Medical College of Georgia, 656 patients were studied with nonstress tests used as the primary test, whereas at the University of Mississippi Medical Center, 614 patients were managed with the nipple stimulation contraction stress stest used as the primary test. Both populations were comparable in regard to pregnancy complications, gestational age, birth weight, perinatal outcomes, cesarean section rates, and the incidence of positive contraction stress tests. Specificity and positive and negative predictive values were similar for both tests. The extended nonstress test had higher sensitivity but also required significantly longer mean duration of testing. The corrected perinatal mortality rate for a reactive nonstress test or negative nipple stimulation contraction stress test was zero within one week of the last test. We conclude that both tests provide excellent primary methods of fetal surveillance and that the decision to use one test rather than the other should be made on the basis of considerations of cost, convenience, time availability, and the presence of specific test contraindications or pregnancy complications. (Am J Obstet Gynecol 1987;157:531-7.)

Key words: Nonstress test, nipple stimulation contraction stress test

During the past decade, antepartum fetal heart rate (FHR) testing has become a principal method of fetal assessment. FHR testing uses two primary approaches, the contraction stress test (CST) and the nonstress test (NST).[1-5] More recently, there has been a trend toward performing the CST by intermittent nipple stimulation (nipple stimulation CST) rather than by the infusion of exogenous oxytocin.[6, 7] Since the inception of both FHR tests, there has been controversy over which test provides better primary evaluation of fetal well-being. One major study, involving 18 institutions during a 4-year period, concluded that the CST was the better primary test.[8] However, this report was flawed in several respects: (1) the individual obstetric features of each study population were not specified; (2) uniform criteria for NST reactivity were not employed; (3) a disproportionate number of patients were studied with CSTs; (4) clinical responses to abnormal test results were not presented; and (5) the NST group had a higher rate of low birth weight infants. In view of these problems, and the increasing popularity of the nipple stimulation CST, we undertook the present study to compare the diagnostic values of the NST and nipple stimulation CST in two comparable obstetric populations, by means of a uniform baseline testing protocol and similar minimum criteria for reactivity.

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Foundation Prize Paper, presented at the Forty-ninth Annual Meeting of The South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 25-28, 1987.

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Material and methods

During an 18-month period, 1270 high-risk obstetric patients were tested at the Medical College of Georgia and the University of Mississippi Medical Center. A common maternal testing protocol was adopted (Table I), incorporating the same minimum criteria for reactivity. At the Medical College of Georgia, all fetuses were tested, with a previously reported NST scheme[9] in which a 30-minute baseline level was established. If at least three accelerations of 15 beats/min and 15 seconds' duration were not present, testing was extended by 30-minute increments until these criteria were met or 90 minutes had elapsed. Any test not producing a 30-minute reactive window was considered nonreactive. An NST was deemed unsatisfactory if a continuous 30-minute recording acceptable for interpretation could not be obtained. Nonreactive 90-minute NSTs were followed by oxytocin-stimulated CSTs (Fig. 1).

At the University of Mississippi Medical Center, the nipple stimulation CST was used as the primary testing

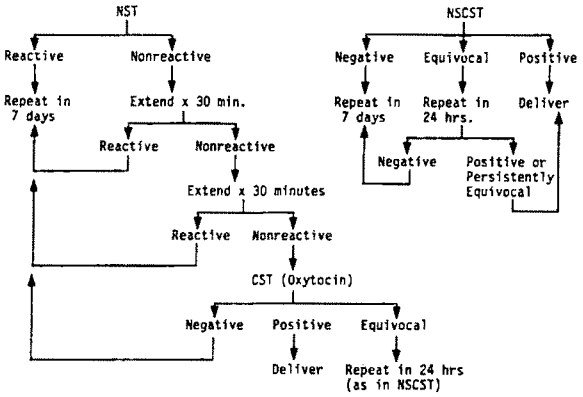


Fig. 1. Management algorithms for the NST (Medical College of Georgia) and nipple stimulation CST(University of Mississippi Medical Center).

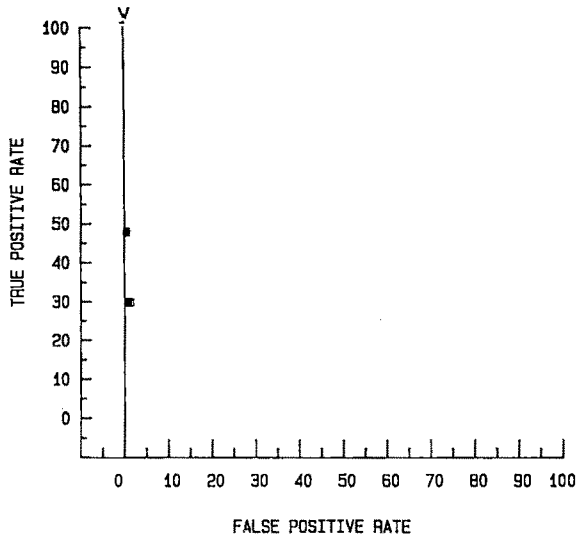


Fig. 2. Receiver operating characteristic curves for the NST *(closed circle)* and nipple stimulation CST *(closed square)*. Line *OY* represents the ideal plot, that is, 100% true positive rate, 0% false positive rate.

Table I. Fetal heart rate testing protocol

A	Baseline period: 30 min
B	Time of day: 0800-1600 hr
C	Maternal dietary state: 2 hr after a meal or 100 gm oral glucose load
D	Maternal drugs: Avoidance of smoking for at least 2 hr and CNS active drugs for at least 8 hr before testing
E	Maternal activity level: Sedentary for at least 1 hr before testing
F	Maternal position: Semi-Fowler's with lateral hip displacement

CNS = Central nervous system.

Table II. Diagnostic test results versus perinatal outcome

	Abnormal outcome	*Normal outcome*	*Total*
Abnormal test	a	c	a + c
Normal test	b	d	b + d
Total	a + b	c + d	a + b + c + d

$$\text{Sensitivity} = \frac{a}{a+b};\ \text{specificity} = \frac{d}{c+d};\ \text{positive predictive value} = \frac{a}{a+c};\ \text{negative predictive value} = \frac{d}{b+d};\ \text{prevalence} = \frac{a+b}{a+b+c+d}$$

Table III. Primary indications for fetal heart rate testing

Diagnosis	*MCG* No.	(%)	*UMMC* No.	(%)
Postdates	212	(32.3)	224	(36.6)
Hypertension	198	(30.2)	197	(32.1)
Diabetes mellitus	96	(14.6)	94	(15.3)
Suspected IUGR	60	(9.1)	43	(7.0)
Rh-sensitization	8	(1.2)	3	(0.5)
Poor obstetrical history	15	(2.3)	15	(2.4)
Decreased fetal activity	15	(2.3)	7	(1.1)
Twins	10	(1.5)	7	(1.1)
Suspected anomaly	14	(2.1)	7	(1.1)
Miscellaneous obstetrical problems	5	(0.8)	3	(0.5)
Miscellaneous medical problems	23	(3.5)	13	(2.1)
Totals	656	(100.0)	614	(100.0)

MCG = Medical College of Georgia; UMMC = University of Mississippi Medical Center; IUGR = intrauterine growth retardation.

$\chi^2 = 13.16$; df = 10; p = 0.215.

method and was performed according to the technique of Huddleston et al.[6] During the baseline period of 30 minutes an NST, with the same minimum criteria used for reactivity as at the Medical College of Georgia, was performed in conjunction with nipple stimulation CST when sufficient uterine activity was not present to perform a spontaneous CST. During this period, the CST by nipple stimulation or by spontaneous contractions was conducted (while the response to fetal movements was monitored). The assessment of fetal reactivity, if not initially present, was continued for at least 30 minutes in each case whether or not uterine activity was elicited. A nipple stimulation CST was considered satisfactory once an interpretable FHR pattern with at least three adequate contractions every 10 minutes was achieved. A nipple stimulation CST was unsatisfactory if an adequate contraction pattern could not be achieved in 30 minutes. Hyperstimulation was defined

Table IV. Perinatal outcomes of study groups

	MCG (N = 613)	*UMMC (N = 582)*	*p Value*
Gestational age (wk)	38.7 ± 3.7 (range 28-43)	38.4 ± 3.4 (range 26-43)	0.65*
Birth weight (gm)	3195 ± 610 (range 660-440)	3094 ± 496 (range 704-4160)	0.45*
Perinatal deaths	4	4	0.80†
Fetal distress	25	36	0.11†
5-Min Apgar <7	18	18	0.82†
Intrauterine growth retardation	34	32	0.45†
Postmaturity	15	21	0.08†
Primary cesarean section	105	82	0.15†

Values for gestational age and birth weight are means ± SD.
*t test, two-tailed.
†z test, two-tailed.

Table V. Outcomes of fetal heart rate tests

	MCG	*UMMC*	*p Value*
Total No. tested	656	614	—
Total No. of tests	1566	1485	—
Tests per patient	2.4 ± 1.4 (range 1-15)	2.5 ± 1.3 (range 1-17)	
No. of patients delivered within 7 days of last satisfactory test	613	582	0.37*
Test duration (min)	36.4 ± 14.8	31.0 ± 3.4	<0.0001*
Last FHR test			
Total reactive	577	500	0.00005†
Total nonreactive	36	82	0.00005†
Total negative CST	3‡	557	—
Total positive/equivocal CST	33‡	25	0.37†

Values of tests per patient and test duration are means ± SD.
*t test, two-tailed.
†z test, two-tailed.
‡CST performed only after nonreactive NST.

as the occurrence of uterine tetany or tachysystole. A nipple stimulation CST was positive if late decelerations occurred with at least 50% of contractions, negative if no late or variable decelerations occurred, and equivocal if occasional late or variable decelerations were present. Clinical management of the various test outcomes is presented in Fig. 1.

Perinatal outcomes, chosen as end points in this study, included death, intrapartum fetal distress, 5 minute Apgar scores, intrauterine growth retardation, and postmaturity syndrome. Correlations of perinatal outcome and test results considered only patients delivered within the week of their last test and were based on the last test before delivery. Diagnostic parameters of sensitivity, specificity, predictive value, and prevalence are defined in Table II. For purposes of comparison, positive CSTs and persistently equivocal CSTs were grouped together because their clinical management was the same. Statistical comparisons used t tests, z tests for significant differences between two proportions, χ^2 tests, and the receiver operating characteristic curve[10] where appropriate.

Results

Test populations. A total of 1270 patients undergoing 3061 FHR tests were studied at the two institutions. Primary test indications are listed in Table III and were similar in both groups. Perinatal outcomes of each testing group are compared in Table IV and were similar for each end point.

Test results and perinatal outcomes. Table V compares the NST and nipple stimulation CST results. Both groups were similar for the total number of tests, the mean number of tests per patient, the proportion of patients delivered within 7 days of their last test, and the percentage of patients having abnormal CSTs. The NST group required significantly more time per test than the nipple stimulation CST group (36.4 versus 31.0 minutes), whereas the nipple stimulation CST group had a significantly greater proportion of nonreactive tests.

The associations of perinatal outcomes and test results are summarized in Table VI. There were no significant differences for any of the major outcome categories when reactive NSTs (Medical College of Geor-

Table VI. Diagnostic values of NST and nipple stimulation CST

	Outcome		
	Abnormal	*Normal*	*Total*
NST			
Nonreactive	33	3	36
Reactive	36	541	577
Total	69	544	613
Nipple stimulation CST			
Positive	20	5	25
Negative	47	510	557
Total	67	515	582

Sensitivity: $\frac{33}{69}$ versus $\frac{20}{67}$; $z = 2.17$; $p = 0.03$.

Specificity: $\frac{541}{544}$ versus $\frac{510}{515}$; $z = 0.728$; $p = 0.46$.

Positive predictive value: $\frac{33}{36}$ versus $\frac{20}{25}$; $z = 0.869$; $p = 0.30$.

Negative predictive value: $\frac{541}{577}$ versus $\frac{510}{557}$; $z = -0.855$; $p = 0.39$.

Prevalence: $\frac{69}{613}$ versus $\frac{67}{582}$; $z = -0.109$; $p = 0.91$.

gia) were compared with negative nipple stimulation CST (University of Mississippi Medical Center) or when nonreactive NSTs (Medical College of Georgia) were compared with positive nipple stimulation CST (University of Mississippi Medical Center) (Table VII). Incidence of bad outcomes was virtually identical in both groups (NST, 11.3%; nipple stimulation CST, 11.5%; $z = -1.09$; $p = 0.91$). Although the NST group had higher sensitivity, specificity, and predictive values, only the differences in test sensitivity proved to be significant by z tests. The receiver operating characteristic curve is another means of evaluating diagnostic tests.[10] The plots derived from both study groups are shown in Fig. 2 and reveal that both fall along the ideal line for diagnostic tests.

There were four perinatal deaths in each group. In the NST group, there was one antepartum death. This occurred after nonreactive NST, which was followed by equivocal results of a CST given to an infant with severe growth retardation at 35 weeks' gestational age. A repeat test was planned for the next day; however, the patient failed to keep her appointment and returned 6 days later with an intrauterine fetal death. Three neonatal deaths occurred in the NST group: one after a nonreactive NST and positive CST in an extremely premature infant weighing 660 gm and two after reactive NSTs (abruptio placentae with perinatal asphyxia and renal dysplasia with neonatal renal failure). In the nipple stimulation CST group, there were two antepartum deaths: one after a negative CST resulting from abruptio placentae and one after a reactive positive CST associated with intrauterine growth retardation. Both neonatal deaths in this group occurred after nonreactive positive CSTs: one with extreme prematurity (weighing 704 gm) and the other after meconium aspiration syndrome in a growth-retarded fetus. None of the perinatal deaths occurring after normal tests (reactive NST or negative CST) could be considered preventable.

Perinatal morbidity was similar in both groups for both reactive NSTs and negative CSTs. In the NST group, 9 of 34 cases could be considered nonpreventable or nonpredictable as they were either consequences of intrapartum accidents or of prematurity. In the nipple stimulation CST group, 11 of 41 cases could be considered nonpreventable or nonpredictable for the same reasons.

Comment

This is the first prospective study comparing the efficacy of the NST with that of the nipple stimulation CST. Unlike the earlier multi-institutional report,[8] we used a standardized maternal state protocol, a uniform initial baseline interval, and common minimum criteria for reactivity. Moreover, both study groups had similar testing indications, obstetric characteristics, and perinatal outcomes. Finally, abnormal test results led to active intervention, usually resulting in delivery within the next 24 hours.

With the use of the same exclusion criteria as those of Freeman et al.,[8] there were no perinatal deaths in our study groups after either a reactive NST or negative nipple stimulation CST. The prevalence of bad outcomes, test specificity, and predictive values was similar for both testing schemes when all major perinatal complications were considered. Test sensitivity was somewhat greater in the NST group, even when corrected for nonpreventable bad outcomes. Although this difference between the two groups was significant, in neither group did test sensitivity exceed 50%. The differences in the incidences of nonreactive tests (Table V) could be explained by the fact that the Medical College of Georgia patients with initially nonreactive 30-minute tests had their sessions extended by 30-minute increments, with those in the University of Mississippi Medical Center group completing their testing in 30 minutes if the nipple stimulation CST was negative whether or not reactivity had been achieved. As reported earlier,[11] a 30-minute FHR baseline NST is associated with a "false" nonreactivity rate in 16% of normal fetuses. Therefore, this difference probably reflects the effect of increased observation time in the Medical College of Georgia group. Conversely, the incidence of positive or persistently equivocal CSTs was quite similar in both groups, suggesting that the extended NST is an effec-

Table VII. Comparison of major perinatal problems versus test outcome

	Perinatal death		*Fetal distress*		*5 Min Apgar score <7*		*IUGR*		*Postmaturity*	
Reactive NST (N = 577)	2		18		7		20		9	
Nonreactive NST (N = 36)	2	$p < 0.001$	16	$p < 0.0001$	11	$p < 0.0005$	14	$p < 0.0001$	6	$p < 0.001$
Negative CST (N = 557)	1		19		8		20		16	
Positive CST (N = 25)	3	$p < 0.001$	17	$p < 0.0001$	10	$p < 0.0005$	12	$p < 0.001$	5	$p < 0.001$

There were fetuses in each group with more than one perinatal complication.

Comparisons of reactive NST versus negative CST and nonreactive NST versus positive CST revealed no significant differences by Fisher's exact test.

tive screening tool for those fetuses requiring further evaluation although the mean length of testing is significantly greater. Finally, the utility of both tests could be examined by comparing those fetuses for whom the initial test provided satisfactory data for assessment. In the nipple stimulation CST group, hyperstimulation was uncommon (28 of 1495 tests), comparing favorably with that reported for the oxytocin CST,[4] whereas 36 tests (2.4%) required exogenous oxytocin. This gave an overall incidence of 4.3% of tests that could not be used for immediate assessment and that necessitated repeated testing later the same day. There were also 75 nipple stimulation CSTs that were initially unsatisfactory, requiring repeat testing sessions. In contrast, there were only 20 of 1566 NSTs (1.3%) that were unsatisfactory at 30 minutes; 10 remained unsatisfactory at 60 minutes; and three were unsatisfactory at 90 minutes.

Antenatal FHR testing, whether by CST or NST, has resulted in reduced rates of perinatal death.[12] Neither method has been clearly shown to provide superior primary fetal surveillance because previous studies have either lacked sufficient numbers for comparison or have not used uniform testing conditions, interpretative criteria, or patient follow-up. The experience of these two high-risk referral centers suggests that the decision to employ one test rather than the other should be made on the basis of specific considerations. These include (1) contraindications to the CST, for example, third-trimester bleeding, (2) fetal risk not related to uteroplacental insufficiency, for example, malformations, (3) gestational age at which uterine insensitivity to oxytocin may preclude a satisfactory CST or at which reactivity criteria are not well established, and (4) cases in which cord vulnerability is suspected, for example, oligohydramnios. A further consideration that may influence test selection is that extending the NST increases the mean length of testing periods and personnel time. This may over time reduce the testing capability of a large obstetric unit treating a substantial volume of high-risk patients.

Our study has shown that, regardless of testing approach, antepartum assessment with FHR testing is capable of providing high specificity and high positive and negative predictive values whereas the sensitivity of either testing method is considerably lower. This is also a shortcoming of most previous reports[13] and reflects unanticipated perinatal problems, for example, abruptio placentae, intrapartum infection, prematurity, as well as the reluctance to set a cut-off point that would incorrectly classify a large number of normal fetuses. It is conceivable that ancillary assessment measures, such as multiple-parameter biophysical testing,[14, 15] could help to resolve this problem, although this remains to be convincingly demonstrated. Furthermore, biophysical testing is inherently more expensive in both equipment and personnel requirements, more time-consuming, and not available in all testing units. It is possible that further refinement of FHR testing, by the inclusion of other information such as baseline rate and variability[16] should be considered by those studying this problem in the future. Even if such improvements in antepartum testing occur, a residual group of fetuses who produce "normal" antenatal tests will have adverse perinatal outcomes, largely arising from such causes as previously mentioned. These fetuses usually elude antenatal detection as their problems occur acutely during the peripartum period. Therefore, close, continuous intrapartum fetal monitoring should be considered mandatory for all high-risk patients, regardless of the results of their antepartum FHR tests.

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Discussion

DR. ALLAN G. W. MCLEOD, Miami, Florida. This is an interesting study from several standpoints. First, that anyone could get two university departments to agree to a similar protocol is a noteworthy achievement. Second, the manuscript is well written and contains a profusion of statistical data, but at times the latter seems somewhat overpowering. In fact, I went back and reread the title of the paper having questioned that I had mistakenly received a copy of a new biomedical statistics test prepared for the subspecialty Board in Maternal-Fetal Medicine. Not being a biomedical statistician I do not plan to comment further on the techniques of statistical analysis, which appeared to be thorough and detailed. As a clinician, however, I am left with a lingering reservation in my own mind as to whether the numbers involved are indeed large enough to allow us to compare perinatal death in the two groups. Third, I was impressed by the striking similarity of the perinatal problems in the two centers, although perhaps I should not have been since our own problems are comparable.

The common maternal testing protocol requiring performance of the test between 0800 and 1600 hours, 2 hours after a meal or glucose load, and without smoking for 2 hours would present major difficulties in a busy obstetric service such as ours at Jackson Memorial Hospital, where in the month of September alone we had 1206 deliveries.

Perinatal death, intrapartum fetal distress, and 5 minute Apgar scores certainly qualify as end points but I would question the decision to classify intrauterine growth retardation or postmaturity syndrome as an end point. Those would appear to be conditions that merit performance of the studies in question.

To have had only four adverse outcomes in each group reflects well on the quality of care of high-risk patients in both centers.

On reviewing the perinatal deaths in the NST group more closely, failure of the testing mechanism should not be attributed to failure of a patient to return as instructed; however, one might question the wisdom of allowing a patient to go home whose fetus showed severe intrauterine growth retardation at 35 weeks and in whom a nonreactive NST and equivocal CST had been obtained. (The "retrospectoscope" is a marvelous instrument.) It is also hard to believe that the three neonatal deaths could have been prevented.

In the nipple stimulation CST group it would appear that a positive test should not be ignored or action deferred even when it is "reactive," especially in the presence of intrauterine growth retardation. We still appear to have difficulty in interpreting the results of some tests and one certainly has to question the validity of the tests, especially the NST in the presence of a very immature fetus.

The fact that out of 1566 nonstress tests only 10 remained unsatisfactory at 60 minutes and only three were unsatisfactory at 90 minutes shows that the extended NST can have a role even in a busy unit.

The authors appear to have shown that the extended NST and nipple stimulation CST are potent weapons in our armamentarium for antepartum screening and that the decision to use one as opposed to the other should be made on the basis of specific considerations or complications of the pregnancy. The authors have outlined limitations of the techniques and have emphasized that their study is but one small step through the quicksand of antepartum fetal testing.

This article has stressed the fact that we must all recognize the importance of adequately counseling our patients to the extent that they fully understand as well as we do that a "normal" antenatal test does not guarantee that the baby is no longer at risk. They must understand that an adverse outcome can still occur, particularly during the peripartum period; hence the need for careful intrapartum monitoring in all high-risk pregnancies irrespective of the results of previous antepartum FHR tests.

The authors must be congratulated for their cooperative effort to shed a little more light in this complicated field on a prospective basis.

DISCUSSER NO. 2. I would like to make one comment that affects many of us. In private practice we are able to do NSTs easily in the office whereas the nipple stimulation test would require a hospital setting in the event of tetanic contractions.

DR. DEVOE (Closing). Dr. McLeod really has left little to respond to. I thought he was extraordinarily kind but I found The Homestead to be a very hospitable environment. I think the points that he emphasized

were the very messages we were trying to get across. One, and the one foremost, is to recognize that all testing schemes have limitations. Second, retrospectively you always criticize yourself for allowing a patient to leave the unit who perhaps should have been delivered. We all contend with difficult patients and this I think is an obstetric problem that will continue indefinitely. Finally the issue is that we should be cautious about trying to overreach the diagnostic ability of a test. Test sensitivity, which should be an ideal measure of how good a screening test is, really reflects how likely you are to predict an outcome which is going to happen sometime in the future when that future is still far from the time the test was done. The point made about where the test is performed is a crucial one. When we are doing contraction stress testing at MCG, we do it in an environment of the labor and delivery area. Tetanic contractions can occur with either testing approach and of course, with our approach, we have already selected the babies who are at the highest risk for having an abnormal CST. That makes it much more inconvenient for the practitioner whose labor and delivery setting might not be proximal to the office, although it is safer for the patient.

Ovarian prostaglandin synthase: Immunohistochemical localization in the rat

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Ovarian prostaglandin synthase is stimulated by the luteinizing hormone surge resulting in the preovulatory increase in prostaglandins. In the present study, the ovarian cellular localization of prostaglandin synthase was identified by immunohistochemistry. Ovaries were collected from 27-day-old rats at the time of stimulation with pregnant mare serum gonadotropin (8 IU) (zero hours), 48 hours later, or 8 hours after administration of human chorionic gonadotropin (5 IU). At zero hours prostaglandin synthase immunostaining was present in the theca of larger follicles and interstitial regions. The number and intensity of immunostained cells in the theca increased between zero and 48 hours. The granulosa cell layer adjacent to the basement membrane in large antral follicles exhibited immunostaining. A similar staining pattern was observed 8 hours after human chorionic gonadotropin. These observations indicate that in the rat, the theca, interstitium, and granulosa contain prostaglandin synthase immunoreactivity and may contribute prostaglandins during follicular development and ovulation. (Am J Obstet Gynecol 1987;157:537-43.)

Key words: Prostaglandins, prostaglandin synthase, ovary, follicle, ovulation

Prostaglandins (PGs) act as important intercellular mediators of hormone action. In the ovary, the preovulatory increase of luteinizing hormone (LH) induces a fivefold increase in the activity of PG synthase, the enzyme that regulates the rate-limiting step in PG synthesis. The stimulation of PG synthase activity by LH results in an increase of ovarian PG levels that reach a maximum at the time of ovulation.[1] The preovulatory increase in PGs appears to be essential for follicular rupture to occur according to results of studies in which PG synthesis has been inhibited and ovulation is blocked.[1,2] The mechanisms by which PGs control this rupture, however, are uncertain. It has been proposed that PGs promote smooth muscle contractility in the follicular wall, resulting in rupture of the follicular apex already weakened by proteolytic enzymes.[1,2] PGs may also act on the vasculature to influence ovarian hyperemia.

Although the hormonally mediated control of PG production has been extensively investigated, the cellular localization of ovarian PG synthase has received little attention. The development of a monoclonal an-

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tibody to PG synthase in our laboratory has provided a unique probe necessary to identify the cells containing PG synthase and to further characterize the action of LH on enzyme synthesis and activity.[3] The PG synthase antibody has been used to demonstrate a threefold increase in PG synthase protein after stimulation with human chorionic gonadotropin (HCG). These findings indicate that the HCG-stimulated increase in preovulatory PGs results in part from de novo synthesis of the enzyme, a question that previously could not be addressed with existing methodologies.[3] The purpose of the present study was to examine the ovarian cells containing PG synthase by immunohistochemical techniques. Cellular identification of PG synthase was accomplished by first optimizing the immunohistochemical staining in a known positive control tissue, kidney,[4] and subsequently identifying the cells containing PG synthase in the rat ovary.

Methods

Animals. Immature female rats (Sprague-Dawley, Charles River) were housed under controlled environmental conditions with 14 hours of light and given free access to water and rat chow. At 27 days of age, animals were primed with 8 IU of pregnant mare serum gonadotropin (PMSG) between 0900 and 1100 hours to stimulate follicular development. Fifty-two hours after administration of pregnant mare serum gonadotropin, rats were injected with 5 IU of HCG to initiate ovulation. Animals were killed and postmortem examinations performed at the time of pregnant mare serum gonadotropin priming (zero hours), 48 to 51 hours later (48 hours), or at 8 hours after HCG administration (8 hours HCG). The ovaries and kidneys were removed, cleaned of adnexa, and embedded and immunostained as described later.

Tissue preparation. Tissues were immersion-fixed as described later, dehydrated, and embedded in paraffin. The effect of different fixatives on preservation of antigenic integrity was tested by the use of 10% neutral buffered formalin, Bouin's solution, paraformaldehyde (1% to 4%), and/or glutaraldehyde (0.25% to 4%). Tissues were serially sectioned (5 μm) and PG synthase localized as described later.

PG synthase antibody. Monoclonal antibodies to PG synthase purified from sheep seminal vesicles were prepared as described previously.[3] Briefly, PG synthase was purified from sheep seminal vesicle membranes by chromatography on diethylaminoethyl cellulose and Lentil Lectin Sepharose. Balb/c mice were immunized with 50 μg of PG synthase, screened for antibody production by immunoprecipitation and enzyme immunoassay, and splenic lymphocytes fused to SP2/O myeloma cells. Hybridoma medium containing PG synthase–specific antibodies, as determined by enzyme-linked immunosorbent assay and immunoblots, was used for immunohistochemistry.

Immunohistochemistry. Immunohistochemistry was performed with the Vectastain avidin-biotin complex (ABC) peroxidase procedure (Vector Laboratories, Burlingame, Calif.) as described by Hsu et al.[5] The endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 20 minutes. Tissue sections were rinsed with phosphate-buffered saline (10 mmol/L sodium phosphate, 4 mmol/L potassium phosphate, 0.15 mol/L sodium chloride, pH 7.4) before treatment with 1% normal swine serum for 30 minutes to reduce nonspecific background staining. Excess serum was blotted and the sections were sequentially incubated with anti-PG synthase (1:20) for 30 minutes, biotinylated rabbit antimouse IgM (1:600) for 30 minutes, and preformed ABC (1:160) for 45 minutes. After each step, the sections were washed with phosphate-buffered solution (three changes for 2 minutes each). Peroxidase activity was demonstrated by incubation in 0.05% 3,3′-diaminobenzidine containing 0.03% H_2O_2. The sections were rinsed with water, dehydrated through ascending concentrations of ethanol, immersed in xylene, and coverslipped. The slides were examined by bright field and Normarski interference light microscopy.

The immunohistochemical specificity was examined by substitution of the primary antibody with phosphate-buffered solution, normal swine serum (1:10), or a monoclonal progesterone antibody, or by omitting the secondary antibody. In addition, the primary antibody was preabsorbed with PG synthase (10 μl of antibody: 90 μl [45 μg] of antigen) for 1 hour at 22° C before being processed for immunohistochemistry.

Morphometric analysis. Quantitative evaluation of ovarian immunostaining was obtained by means of a grid overlay method as described by Weibel and Bolender.[6] Ovaries were serially sectioned at 5 μm and on every fifteenth section a grid containing 42 points was placed over follicles containing an oocyte. The percentage of points falling on a stained cell in the theca, interstitial, or granulosa cell compartments versus the total number of points counted for that cell type (stained and unstained) were tabulated. The relative immunostaining intensity of the theca, interstitium, and granulosa was scored on a 0 to 3 scale where 0 represents no staining and 3 indicates a maximum staining. Follicular diameter was determined by two measurements taken at right angles.

Statistics. An analysis of variance was used to test differences between the mean number of stained cells in the theca or granulosa in follicles of the same diameter collected at different treatments (i.e., times after pregnant mare serum gonadotropin or HCG administration) or in follicles of varying size within the same

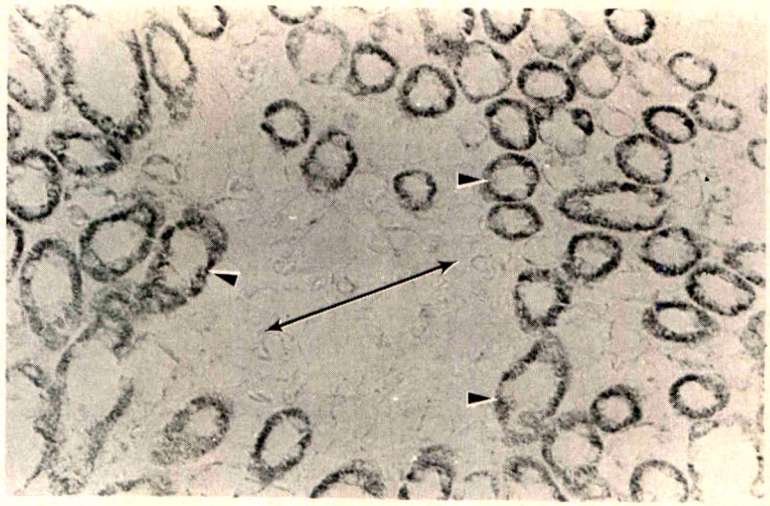


Fig. 1. Photomicrograph of the medullary region of the rat kidney immunostained for prostaglandin (PG) synthase. PG synthase is identified by the dark diaminobenzidine reaction product present in the collecting tubules *(arrowheads)*. Adjacent areas with the loops of Henle and vasa recta are unstained *(arrows)*.

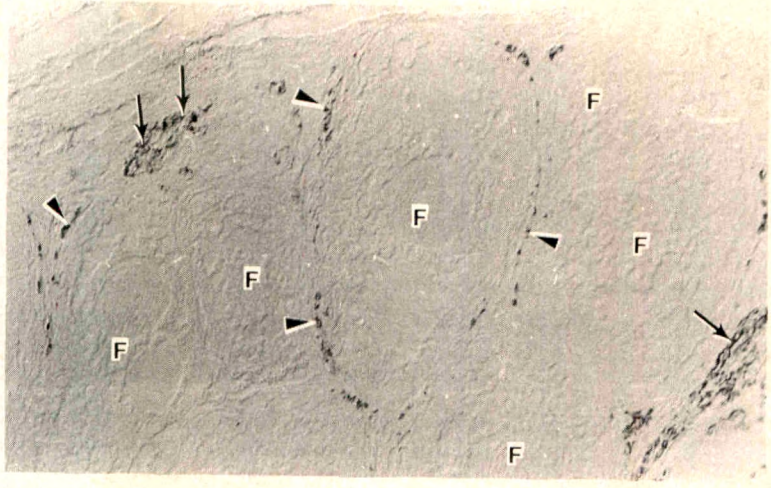


Fig. 2. Photomicrograph of an ovary from a 27-day-old rat before stimulation with pregnant mare serum gonadotropin immunostained for prostaglandin synthase. Staining is present in the theca *(arrowheads)* of antral follicles and the interstitium *(arrows)*. *F* = follicle.

treatment or in the interstitium at different treatments. If significance was obtained with analysis of variance, the Duncan multiple range procedure was used to test for group comparisons.[7]

Results

Effect of fixation on PG synthase immunostaining. Superior preservation of the antigen was observed in tissues fixed in 2% to 4% paraformaldehyde and 10% neutral buffered formalin. Fixation of kidney tissue in either paraformaldehyde or formalin resulted in immunostaining of the collecting tubules in the medullary regions (Fig. 1). The loops of Henle were unstained. Immunohistochemistry of kidney tissue fixed in Bouin's solution demonstrated a similar pattern of staining as observed for paraformaldehyde and formalin with the exception that a general, diffuse, connective tissue staining was present. The addition to 4% paraformaldehyde of glutaraldehyde in concentrations as low as 0.25% diminished the immunoreactivity of PG synthase staining.

In ovarian tissue, a similar paradigm for the effect of fixation on immunostaining was observed. Fixation in either 2% to 4% paraformaldehyde or formalin resulted in superior antigen preservation compared with that obtained in either Bouin's solution or parafor-

Table I. Prostaglandin synthase localization in the theca and granulosa cell layers during different stages of follicular development

Follicle size (μm diameter)	*Sample**	*Theca*		*Granulosa*	
		%†	*Relative score‡*	*%*	*Relative score*
0-100	0 hr	2 ± 1^{a}	1	0^{a}	0
	48 hr	$12 \pm 3^{a,b}$	1	0^{a}	0
	8 hr HCG	34 ± 3^{c}	2	0^{a}	0
101-200	0 hr	15 ± 2^{b}	1	0^{a}	0
	48 hr	$45 \pm 2^{d,e}$	2	0^{a}	0
	8 hr HCG	49 ± 3^{e}	2	3 ± 1^{a}	1
201-300	0 hr	$37 \pm 3^{c,d}$	2	1 ± 1^{a}	1
	48 hr	64 ± 2^{g}	2	0^{a}	0
	8 hr HCG	$67 \pm 3^{f,g}$	2	0^{a}	0
301-400	0 hr	$43 \pm 4^{c,d,e}$	2	1 ± 1^{a}	1
	48 hr	72 ± 3^{g}	2	1 ± 1^{a}	1
	8 hr HCG	67 ± 3^{g}	2	1 ± 1^{a}	1
>401	0 hr	48 ± 6^{e}	2	3 ± 3^{a}	1
	48 hr	$55 \pm 7^{e,f}$	2	14 ± 3^{b}	2
	8 hr HCG	69 ± 6^{g}	2	9 ± 2^{b}	2

*0 hr = ovaries collected at the time of priming with PMSG. N = 5 ovaries with an average of 8 sections per ovary and 39 follicles per ovary examined. 48 hr = ovaries collected 48 to 51 hr after priming with PMSG. N = 4 ovaries with an average of 12 sections per ovary and 70 follicles per ovary counted. 8 hr HCG = ovaries collected 8 hr after administration of HCG to PMSG–primed animals. N = 3 ovaries with an average of 10 sections per ovary and 52 follicles per ovary analyzed.

†Percentage represents number of immunostained cells compared to the total number of cells counted. Values within the theca or granulosa with different superscripts are significantly different, $p < 0.05$.

‡Relative score = staining intensity graded on a 0 to 3 scale where 0 = no staining and 3 = maximum staining.

maldehyde with glutaraldehyde. All of the studies localizing ovarian PG synthase at different stages of follicular development were performed on tissues fixed with 4% paraformaldehyde.

Ovarian PG synthase localization. In ovaries collected at the time of priming with pregnant mare serum gonadotropin (zero hours; N = 7 animals, N = 5 ovaries morphometrically analyzed, 39 follicles per ovary), PG synthase was present in the theca of the follicles >100 μm diameter and in the interstitial regions (Fig. 2). The number of stained cells in the theca increased as the follicle size increased ($p < 0.01$) up to 300 μm in diameter (Table I). In the interstitial regions, 62% ± 2% of the cells exhibited intense immunoreactivity. Immunostaining was seldom observed in the granulosa cell layer regardless of follicular diameter (Table I).

A similar pattern of immunostaining was present in ovaries collected 48 hours after administration of pregnant mare serum gonadotropin (N = 7 animals, N = 4 ovaries morphometrically analyzed, 70 follicles per ovary). PG synthase was observed in the theca and the number and intensity of stained cells increased ($p < 0.01$) with increasing follicular size up to 300 μm (Table I). Intense staining was present in 72% ± 2% of the cells in the interstitial region (Fig. 3). The granulosa layer was almost absent of cells containing PG synthase in follicles less than 400 μm in diameter. In 75% of the follicles greater than 400 μm in diameter, the mural granulosa cells contained PG synthase immunoreactivity (Table I).

In ovaries collected 8 hours after administration of HCG (N = 8 animals; N = 3 ovaries analyzed morphometrically; 52 follicles per ovary), the theca, granulosa, and interstitial regions displayed PG synthase staining similar to ovaries collected 48 hours after stimulation with pregnant mare serum gonadotropin (Table I). In the interstitium 66% ± 2% of the cells exhibited intense immunostaining. Granulosa cells adjacent to the basement membrane exhibited a punctate staining pattern (Fig. 4). Ovarian sections incubated with preabsorbed antibody, with swine serum, or with a monoclonal progesterone antibody did not exhibit immunostaining (Fig. 5).

Comment

In the present study, a monoclonal antibody to PG synthase was used to identify the cellular origin of the enzyme in the rat ovary. The demonstration of ovarian PG synthase reflects the cellular content of immunoreactive enzyme and is not an index of PG production by the various ovarian compartments. The immunohistochemical localization of PG synthase was first optimized in kidney where the cellular origin of the enzyme has previously been reported.[4] Smith and Wilkin[4] observed immunofluorescence present in the collecting tubules of the kidney medulla and the interstitial cells of the renal papilla. In the present study, PG synthase

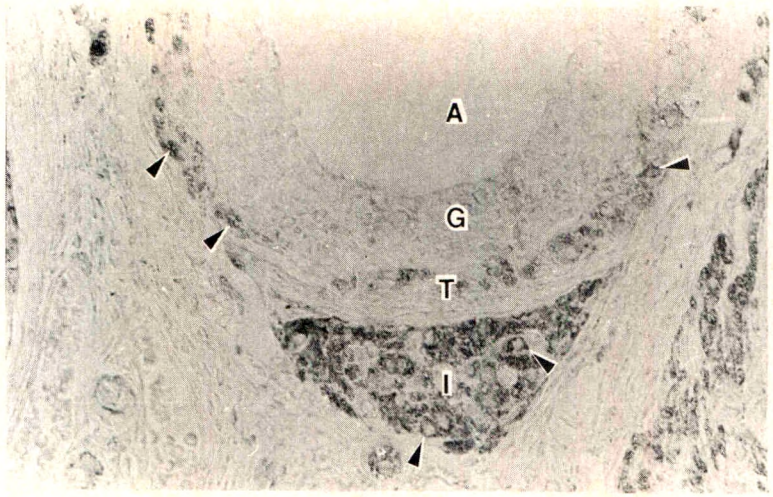


Fig. 3. Photomicrograph of an ovary collected 48 hours after stimulation with pregnant mare serum gonadotropin and immunostained for prostaglandin (PG) synthase. PG synthase staining *(arrows)* is present in the interstitium *(I)* and theca *(T)*. Granulosa cell layer *(G)* is unstained. *A* = antrum.

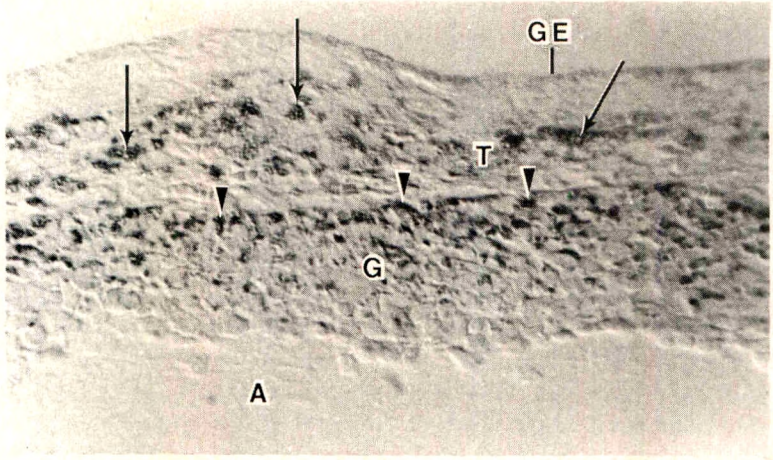


Fig. 4. Photomicrograph of a preovulatory follicle from an ovary collected 8 hours after administration of human chorionic gonadotropin and immunostained for prostaglandin synthase. Staining is present in the mural granulosa cells *(G, arrowheads)* and theca *(T, arrows)*. *A* = antrum; *GE* = germinal epithelium.

staining after fixation in paraformaldehyde exhibited a similar pattern of immunostaining in the rat kidney. Background staining of the connective tissue was observed in tissues fixed in Bouin's solution and antigenicity was destroyed by fixation in glutaraldehyde. Smith and Rollins[8] also reported superior PG synthase preservation with paraformaldehyde fixation and abolition of antigenicity with glutaraldehyde.

Although the hormonally mediated control of ovarian PG production has been studied,[1] the cells that contain PG synthase and consequently are responsible for PG production have not been positively identified. The sites of PG synthesis have been inferred by biochemical analysis of PG levels in separated ovarian compartments. In the rabbit, the granulosa cell layer has previously been reported to produce 2 to 4 times more PGF than cultured thecal tissue.[9] Triebwasser et al.[10] observed that cultured rabbit granulosa cells produced five times more PGE and nine times more PGF than the cultured residual wall of preovulatory Graafian follicles, suggesting that the granulosa is the major site of PG synthesis. In the human, both the theca and the granulosa have been reported to produce substantial amounts of PGs in culture.[11, 12] Patwardhan and Lanthier[12] found that, on a per follicle basis, the theca demonstrated a greater capacity to produce PGs in vitro

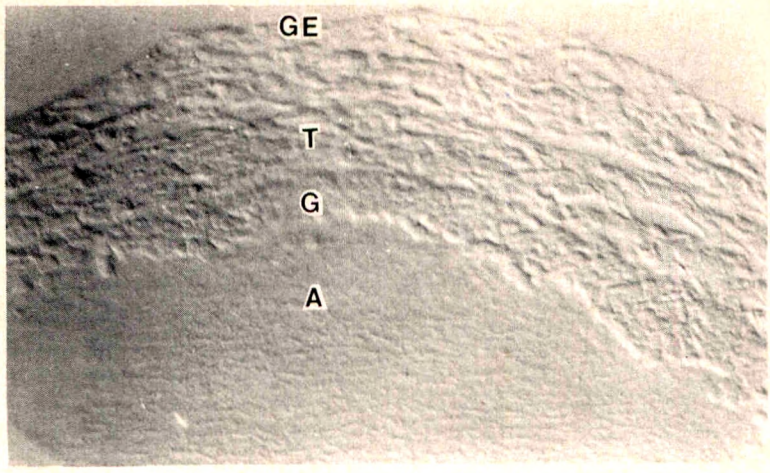


Fig. 5. Photomicrograph of a preovulatory follicle from an ovary collected 8 hours after administration of human chorionic gonadotropin and immunostained for prostaglandin (PG) synthase. The antibody was preabsorbed with PG synthase before immunostaining. Immunostaining was absent. *A* = antrum; *G* = granulosa; *GE* = germinal epithelium; *T* = theca.

than the corresponding granulosa cells. However, thecal PG production was not stimulated by gonadotropins.

Isolated rat granulosa cells respond to an LH stimulation with a significant increase in PG production[1] and this ovarian compartment has been proposed as the predominant source of PG synthase in the rat. In contrast to these findings, Lindner et al.[2] observed that, in follicles isolated from proestrus rats, the follicle wall produced approximately fourfold more PGE than an equivalent number of granulosa cells. The observations from the present study, which quantitate immunoreactivity instead of enzyme activity, support the role of the theca and surrounding interstitial cells in the production of ovarian PGs. PG synthase was present in the theca of larger antral follicles in ovaries collected from animals before exogenous hormonal stimulation. Of particular interest was the increase in number and intensity of immunostained cells in the theca after administration of pregnant mare serum gonadotropin, indicating a hormonal regulation of thecal PG synthase. Thecal PGs may modulate early follicular development since PGE_2 can mimic the action of follicle-stimulating hormone (FSH) on granulosa cells and of LH on thecal cells.[11] PGE produced by the theca may diffuse across the basement membrane and assist in the induction of FSH receptors and FSH responsiveness of granulosa cells in immature follicles. In addition, PGs may act in an LH-like manner on the theca of adjacent follicles assisting in follicular growth.[11] Thus the theca may be an important source of PGs during follicular development in the rat.

The granulosa cells contained PG synthase only in large preovulatory follicles. It is well documented that follicular PGs increase 3 to 5 hours after an LH stimulation.[1, 2] The lag period has been postulated as a period of de novo synthesis of the enzyme. Our observation of PG synthase present in the granulosa cells before HCG administration may represent either the natural sequence of differentiation and maturation of the granulosa cell layer after an FSH-like stimulation, a partial response to exogenous LH present in the pregnant mare serum gonadotropin, or stimulation by endogenous LH. The presence of PG synthase staining in 9 of 12 preovulatory follicles suggests that this is not a spurious observation but may reflect endogenous or exogenous LH action present in the pregnant mare serum gonadotropin–stimulated peripubertal animal. In future studies, PG synthase will be localized during the estrous cycle of adult rats. This paradigm will allow examination of granulosa cell staining before and after the endogenous LH surge in the absence of possible exogenous LH action from treatment with pregnant mare serum gonadotropin.

PG synthase staining in granulosa cells was present in a heterogenous distribution. Immunostaining was distributed only in granulosa cells adjacent to the basement membrane. The mural granulosa cells exhibited a punctate staining pattern, which was markedly different than the pattern observed in the theca and interstitial cells. Our observations are consistent with the concept of a heterogenous granulosa cell population as proposed by other workers examining hormonal stimulation of granulosa cells,[13] cytochrome P-450 activity,[14] and cell surface antigens.[15] For example, Goldring et al.[14] noted a regional variation in cytochrome P-450 activity such that granulosa cells adjacent to the basement membrane had an increase in FSH-stimulated

cytochrome P-450 activity compared with cells located in deeper layers. In addition, FSH initiates differentiation of a surface membrane antigen in only the mural granulosa cells.[15]

In summary, the present results demonstrate for the first time that, with immunohistochemistry, the theca and interstitium of the rat ovary contain PG synthase. The change in thecal immunostaining may reflect follicular differentiation caused by stimulation with pregnant mare serum gonadotropin. The mural granulosa cells of large preovulatory follicles also exhibited an increased quantity of PG synthase. In the rat, the theca, interstitium, and granulosa all appear to contribute to ovarian PGs.

The didactic criticisms of Dr. William LeMaire throughout all stages of this project are gratefully acknowledged. The assistance of Drs. Mehrdad Nadji and Jocelyne Weissman of the Department of Pathology and the technical help of Brad Ress were fundamental during the early stages of immunohistochemical optimization. We appreciate the provision of pregnant mare serum gonadotropin by the National Hormone and Pituitary Program, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

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Evaluation of the atypical Pap smear

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Two hundred thirty-six consecutively referred patients with "atypical" but not dysplastic Pap smears were evaluated by colposcopy and directed biopsies to assess the significance of this ambiguous result. Additionally the histologic diagnosis was compared with the results obtained by repetition of the Pap smear, interpretation of cervigrams, and the visual impression of the colposcopist to evaluate the accuracy of these three modalities as intermediate screening procedures. Fifty-eight patients (25%) had biopsy-proved cervical intraepithelial neoplasia. Repeat Pap smears identified only 17% of these patients. Colposcopists noted atypical transformation zones in 97% of the patients with cervical intraepithelial neoplasia. Cervigrams identified 81% of the cervical intraepithelial neoplastic lesions but had a 15% method failure (uninterpretable). The time-honored tradition of repeating atypical smears before definitive diagnostic procedures are performed is to be condemned. Neither colposcopy nor cervicography was judged to be an ideal intermediate screening procedure, but both were superior to Pap smear repetition. (Am J Obstet Gynecol 1987;157:544-9.)

Key words: Cervical intraepithelial neoplasia, Pap smear, colposcopy, cervicography

Screening Pap smears that are compatible with cervical intraepithelial neoplasia dictate further patient evaluation and establishment of a histologic diagnosis. Patients with cytologically normal screening Pap smears require only continued periodic screening. Other screening smears are variously reported as containing "atypical" cells. Most frequently these cytologic abnormalities can be attributed to recognized inflammatory conditions. Of more concern are those smears that have a significant but nondiagnostic abnormality that cannot be immediately attributed solely to inflammation. Early neoplastic lesions may have minimal cytologic abnormalities. Samples from more advanced lesions may contain only a small number of atypical cells and not be representative of the entire lesion. In other instances the reported atypia may represent diagnostic uncertainty. The next appropriate step in the management of these patients remains unclear.

A critical correlation of these smears with subsequent histologic findings may permit the clinician to assess the significance of these ambiguous smears provided that there is consistency in the evaluation of the cytologic changes and accuracy in the establishment of a histologic diagnosis. Preliminary unreported data in the colposcopy clinic at East Carolina University suggested that nondysplastic screening pap smears interpreted as "class II significant cellular atypia" by the state cytology laboratory were associated with histologic cervical intraepithelial neoplasia and invasive carcinoma in a sufficient number of patients to warrant further evaluation of these patients. Recent studies in other institutions have shown that a significant number of patients with atypical cytologic findings when evaluated are found to have cervical intraepithelial neoplasia.[1, 2]

Because of an increasing number of these patients being seen in our clinic, we additionally evaluated cervicography, simple repetition of the Pap smear, and colposcopy without biopsy as potential intermediate screening methods in these patients.

Material and methods

Two hundred thirty-six consecutively referred nonpregnant patients with class II significant cellular atypia Pap smears were evaluated. Repeat Pap smears were obtained from the endocervix and portio with wooden spatula and a saline-moistened cotton swab. Cervigrams, 35 mm color slides, were made of the transformation zone in the manner described by Stafl.[3] Colposcopy was then performed by one of three faculty members proficient in colposcopy, and directed biopsy specimens of the cervix were obtained. The transformation zone was biopsied in all patients and the histologic diagnosis was established as the reference point for evaluation of the proposed intermediate screening procedures. Endocervical curettage was performed if anything other than native columnar epithelium was present in the endocervical canal.

Pap smears and biopsy specimens were interpreted

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Table I. Correlation between cytologic and histologic findings

Cytologic findings	*Histologic findings*				
	Benign	*CIN I*	*CIN II*	*CIN III*	*Total*
Benign	174	43	4	1	222
CIN I	3	4	2	0	9
CIN II	1	1	1	2	5
CIN III	0	0	0	0	0
Total	178	48	7	3	236

CIN, cervical intraepithelial neoplasia.

independently. Cervigrams were interpreted without knowledge of cytologic or histologic findings by an experienced colposcopist and reviewer of cervigrams who was not otherwise involved in patient evaluation or care.

Cytologic and histologic findings were reported as benign, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, or cervical intraepithelial neoplasia III. Colposcopists were required to identify and report as suspicious transformation zones containing aceto-white epithelium with or without the vascular changes of punctation and mosaicism that, in their estimation, potentially represented cervical intraepithelial neoplasia and warranted biopsy. If the entire transformation zone was not visible, the colposcopy was considered and reported as unsatisfactory.

Cervigrams were interpreted as: (1) transformation zone entirely seen and normal, (2) transformation zone not entirely seen but no abnormalities seen, (3) no transformation zone seen—unsatisfactory, (4) technically defective (inadequate visualization of the cervix), or (5) if aceto-white epithelium with or without associated vascular changes thought to be compatible with cervical intraepithelial neoplasia was visible, the cervigram was considered and reported as suspicious.

If the initial Pap smears obtained in our clinic suggested cervical intraepithelial neoplasia I but the histologic findings we obtained at that initial visit failed to identify cervical intraepithelial neoplasia, the patient was reevaluated by colposcopy, additional biopsies, and Pap smear. If these additional biopsy specimens failed to demonstrate cervical intraepithelial neoplasia and the second Pap smear was benign, the benign histologic diagnosis was accepted. If the initial Pap smear obtained in our clinic suggested cervical intraepithelial neoplasia II or III that could not be confirmed by the colposcopic-directed biopsies obtained at that visit, the patient was similarly reevaluated. If cervical intraepithelial neoplasia could not be confirmed by histologic examination of the additional biopsy specimens in these patients, a conization was performed.

The correlation between the results of each of the proposed intermediate screening procedures and the final histologic diagnosis was made according to the following definitions: sensitivity = true positive/true positive + false negative; specificity = true negative/true negative + false positive; positive predictive value = true positive/true positive + false positive; and negative predictive value = true negative/true negative + false negative.

Results

The mean interval between screening Pap smears and the initial evaluation visit was 4.7 months (range 3 to 52 weeks) and the mean age of the patients was 25 years (range 13 to 63 years). Fifty-eight of the 236 patients (25%) had histologically proved cervical intraepithelial neoplasia. No patients had invasive carcinoma. The correlation between the cytologic findings obtained in our clinic and the histologic diagnosis is displayed in Table I. These Pap smears identified only 10 of the 58 patients (17%) with cervical intraepithelial neoplasia. Only five of the 10 patients with cervical intraepithelial neoplasia II or III were identified by cytologic tests. Four patients whose initial Pap smears in our clinic suggested cervical intraepithelial neoplasia I or II could not be proved by initial histologic findings to have cervical intraepithelial neoplasia. Further evaluations did not identify histologic cervical intraepithelial neoplasia in any of these patients.

The correlation between the cervigram interpretation and the histologic diagnosis is shown in Table II. Cervicography was unsatisfactory in 13 (6%) and technically defective in 22 (9%) of the 236 patients. Excluding these 35 patients, 52 of the remaining 201 patients had cervical intraepithelial neoplastic histologic findings. Forty-seven (90%) of these patients were identified by cervicography. None of the nine patients with cervical intraepithelial neoplasia II or III who had interpretable cervigrams had a negative cervigram result.

The correlation between the visual interpretation of the colposcopist and the histologic finding is shown in Table III. Fifty-six of the 58 patients (97%) with cervical intraepithelial neoplasia were considered to have suspicious colposcopic examinations. Colposcopy was judged to be unsatisfactory in two patients with endocervical cervical intraepithelial neoplasia.

Table II. Correlation between cervicography and histologic findings

Cervicography	*Histologic findings*				
	Benign	*CIN I*	*CIN II*	*CIN III*	*Total*
Negative, entirely seen	29	2	0	0	31
Negative, not entirely seen	61	3	0	0	64
Unsatisfactory	11	1	1	0	13
Technically defective	18	4	0	0	22
Suspicious	59	38	6	3	106
Total	178	48	7	3	236

CIN, cervical intraepithelial neoplasia.

Table III. Correlation between colposcopy and histologic findings

Colposcopy	*Histologic findings*				
	Benign	*CIN I*	*CIN II*	*CIN III*	*Total*
Negative	50	0	0	0	50
Suspicious	128	46	7	3	184
Unsatisfactory	0	2	0	0	2
Total	178	48	7	3	236

CIN, cervical intraepithelial neoplasia.

The correlation between the interpretation of the colposcopist and the interpretation of the cervigram is shown in Table IV. The frequency with which cervical intraepithelial neoplasia was found is shown for each of the possible pairings. Five patients with biopsy-proved cervical intraepithelial neoplasia had cervigrams that were judged to be negative but the transformation zone was not entirely seen. Colposcopists identified three of these five as suspicious. All cervical intraepithelial neoplastic II and III lesions were identified by both techniques provided that the study was satisfactory.

The correlation between the final histologic diagnosis and the result of each of the proposed intermediate screening procedures is shown in Table V. A normal repeat Pap smear was frequently falsely reassuring and a positive repeat smear added no new information. Routine repetition of Pap smears at local health departments significantly delayed referral and definitive diagnostic procedures in this study. Both colposcopy and cervicography were sufficantly sensitive to be considered as potential intermediate screening procedures. In this study cervicography had greater specificity and a higher positive predictive value than did colposcopy.

Comment

There appears to be an increasing number of patients with atypical smears. The proper management has not been defined. In many instances this is a "wastepaper basket" term and the cytologist must describe in detail the interpretation of this entity. Unfortunately, the definition may vary from laboratory to laboratory. Evaluating all patients with atypical smears by colposcopy and directed biopsies exceeds the ability of referral centers to provide this service in many areas. Because 25% of these patients in our study had cervical intraepithelial neoplasia, we feel that further evaluation of patients with atypical smears should be carried out. If an ideal intermediate screening procedure could be identified, the unnecessary use of limited colposcopic services could be avoided without an accompanying compromise of patient care.

In our experience, a repeat Pap smear did not help to identify the patient with cervical intraepithelial neoplasia. The repeat Pap smear most frequently was benign. Only 17% of the patients with cervical intraepithelial neoplasia were identified by the repeat cytologic testing. This prompted a review of both cytologic and histologic material. It became apparent that there was significant variation between pathologists within a single institution and between pathologists in different institutions in the interpretation of mildly atypical histologic changes in the epithelium. Similar variations occurred when cytologic interpretations were subjected to similar internal and external review. It appears that it is extremely difficult to differentiate between reactive changes in immature squamous metaplasia and very early neoplastic changes. With these findings we cannot advocate simple Pap smear repetition as the sole method of evaluation of these patients and the time-honored practice of repeating atypical smears should be abandoned. Other authors have noted a significant

Table IV. Correlation between cervicography and colposcopy

	Cervicography					
Colposcopy	*Negative, entirely seen*	*Negative, not entirely seen*	*Unsatisfactory*	*Technically defective*	*Suspicious*	*Total*
Suspicious	16	43 3 CIN I	10 1 CIN I 1 CIN II	17 4 CIN I	98 38 CIN I 6 CIN II 3 CIN III	184
Negative	15	19	3	5	8	50
Unsatisfactory	0	2 2 CIN I	0	0	0	2
Total	31	64	13	22	106	236

CIN, cervical intraepithelial neoplasia.

Table V. Correlation between satisfactory screening procedures and final histologic diagnosis

Method	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
Pap smear	17	98	71	78
Colposcopy	100	28	30	100
Cervicography	90	60	44	95

false negative rate in repeat Pap smears and have advised against repeating smears to confirm cytologic abnormalities.[4-6]

Colposcopists identified all patients with exocervical cervical intraepithelial neoplasia and the two patients with isolated endocervical cervical intraepithelial neoplasia were considered to have had unsatisfactory examinations. Unfortunately colposcopy did not, in this study, reduce the demand on clinical services because most patients were felt, on the basis of visual inspection, to require biopsy. Two patients with unsatisfactory colposcopic examinations represent a 1% method failure.

Cervicography has limitations when compared with colposcopy. The absence of an exocervical transformation zone resulted in unsatisfactory studies in 6% of the patients. This is probably an irreducible number in this study population. Unsatisfactory studies occurred more frequently, but not exclusively, in elderly patients. Nine percent of the studies resulted in technically defective cervigrams. Because experience was gained during the course of the study, the frequency of technically defective cervigrams decreased. It is important to take as much care to prepare the cervix for cervicography as is done for colposcopy. The combination of unsatisfactory and technically defective studies resulted in a 15% method failure rate. Manipulation of the cervix to expose the endocervical canal and the ability to continuously focus the colposcope makes the method failure rate extremely low with colposcopy. Five patients with biopsy-proved cervical intraepithelial neoplasia I had normal cervigrams. No patients with cervical intraepithelial neoplasia II or III had normal cervigrams. Our experience with cervicography in this setting suggests that this alternative way of screening for abnormalities of the epithelium in the transformation zone is superior to simple Pap smear repetition. If the intent of intermediate screening is to identify only significant lesions, cervicography may permit more efficient use of limited resources. The cervix can be photographed at a remote site and the cervigram reviewed by a competent colposcopist without transportation of the patient to the referral center. This study suggests that advanced cervical intraepithelial neoplastic lesions can be identified by this technology.

In our opinion, the limitations of cervicography make it an unacceptable substitute for colposcopy when the intent is to establish a diagnosis. Cervigrams do provide good photographic documentation of the exocervical transformation zone. Because a small number of patients with cervical intraepithelial neoplasia I were not identified by cervicography, the decision to use this as a intermediate screen in the patient with an atypical Pap smear should be based on local constraints in the health care system. Colposcopy and directed biopsies are the standard by which this technology should be judged. If cervicography is used as an intermediate screen, it should be with the clear knowledge that all patients with unsatisfactory study results must be evaluated further, that technically defective studies must be repeated, and that patients with normal cervigrams should not be considered disease free on the basis of cervicography alone.

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Discussion

Dr. George L. Mayer, Jacksonville, Florida (Official Guest). Dr. Jones and his colleagues have performed a detailed analysis of different courses of evaluation for the class II "atypical, but not dysplastic" Pap smear. They concurrently evaluated, by several methods, 236 consecutive referrals from their North Carolina state cytology laboratory. The goal was to avoid the necessity for definitive colposcopy. The standard for accuracy was pegged to colposcopy with directed biopsy, endocervical curettage, and conization when indicated. The intermediate screening techniques evaluated were: (1) repeat Pap smear, (2) cervicography, and (3) screening colposcopy without biopsy.

Of the 236 patients, 58 were demonstrated to have cervical intraepithelial neoplasia, but only 10 of the 58 (or 17%) had a second Pap smear that was still atypical or had become dysplastic. This was despite a mean interval of 4.7 months between Pap smears. Repeat smears did not detect 43 cervical intraepithelial neoplasia I lesions, four cervical intraepithelial neoplasia II lesions, and one cervical intraepithelial neoplasia III lesion.

These data strongly suggest that Pap smears from the North Carolina state laboratory that are reported as "class II, atypical, but not dysplastic" are obtained from patients whose histologic findings are likely to demonstrate dysplasia 25% of the time. This is clearly a distressing problem. Dr. Jones has indicated, in a personal correspondence, that the laboratory reports 55,000 such Pap smears each year for North Carolina.

If the statistics presented in the article can be extrapolated to encompass the entire 55,000 Pap smears, approximately 11,000 patients could have dysplasias missed by a second Pap smear. How many of these lesions would subsequently be detected by additional smears or would progress to outright cancer is, of course, uncertain.

Some indication of the risks for progression can be estimated by referring to studies that have addressed this problem. One such study by Hall and Walton[1] was published in 1968. In that study 206 patients with histologically proved dysplasias, mostly mild, were followed for up to 14 years. Almost 20% of mild dysplasias progressed to more severe lesions, with 6.2% reaching the level of carcinoma in situ. For more advanced dysplasias the progression rate was much higher. Moderate dysplasias became carcinoma in situ in 12.9%, and severe dysplasias became carcinoma in situ in 29.1% of cases. This was before the practice of combining severe dysplasias and carcinoma in situ into cervical intraepithelial neoplasia III.

In 1964 Stern and Neely[2] concluded that patients with dysplasias have a risk of developing carcinoma in situ more than 1000 times greater than have women without documented dysplasia. The risk for developing invasive cancer of the cervix is 100 times greater than for women without documented dysplasia.

Another concern with missed dysplasias is that the transit times from early to advanced lesions may be faster than many realize. Richart and Barron[3] have estimated from a series of their studies that the mean transit time from mild dysplasia to carcinoma in situ is only 5.8 years, and that from more advanced dysplasias is much faster.

A prevailing practice has been to treat the patient for cervicitis and to perform several Pap smears at relatively frequent intervals after reports of atypia without dysplasia. This is on the basis that with each Pap smear dysplasias become less likely to escape detection. This introduces the uncertainty of patient compliance, making it very important to "know your patient."

A confusing factor has been that the reporting of Pap smears varies from laboratory to laboratory and from pathologist to pathologist. Many gynecologists have learned from experience that a class II Pap smear from one laboratory may be labeled class III in another. The old adage of "know your lab" becomes very important when deciding one's course of evaluation for Pap smears with low-level abnormalities.

Current trends in private practice are making "know your patient" and "know your lab" less obtainable goals. Health maintenance organizations and preferred provider organizations assign and reassign patients as affiliations change. Contractural agreements between third-party payers and laboratories shift regularly in my community. These trends reinforce my agreement with Dr. Jones when he condemns "routine repapping" of "abnormal, but not dysplastic" Pap smears or of Pap smears reported by other laboratories that suggest the same thing.

With regard to the cervicography component of his study, Dr. Jones reports 15% of cervigrams to have been unsatisfactory or technically defective. This compares very favorably with Blythe's report,[4] published in 1985, in which 53.9% of cervigrams were unsatisfactory or technically defective. Stafl,[5] who is the prime proponent of cervicography, reported 19.7% defective or unsatisfactory cervigrams in his 1981 report.

I am curious about the qualifications of the personnel who obtained the cervigrams in the study by Jones et al. If resident physicians obtained the cervigrams, this might explain their success. Jones et al's. rate of defective or unsatisfactory cervigrams would probably be less obtainable if performed in outlying areas for later review by colposcopists. Probably results would more closely parallel Blythe's experience,[4] unless there

was intensive training of those individuals who will obtain the cervigrams. I also suspect that some additional training of colposcopists would be required to make them comfortable interpreting the cervigrams.

Colposcopy has become much more readily available, and most gynecologists perform it regularly. Cervicography is not a technique that is widely available. Efforts to train individuals to perform and interpret results might be better directed to training additional colposcopists. Cervicography is not performed in my community (Jacksonville, Florida) and I have no personal experience with it. I will be very interested to see how this technique evolves over the next several years.

In his comments on screening colposcopy, Jones et al. demonstrated little decreased demand on clinical services. His findings confirmed what most colposcopists experience: "you can almost always find something to biopsy."

In conclusion, the dilemma of atypical, but not dysplastic, Pap smears persists. This study effectively demonstrates that there is not a satisfactory intermediate screening procedure to cut down on the use of colposcopy. Certainly repeat normal Pap smears should not be relied on.

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Dr. Paul B. Underwood, Charlottesville, Virginia. I have two questions. One, Jones et al. mentioned that their patients were up to age 63 years. Knowing that postmenopausal women are notoriously difficult to evaluate colposcopically, did they look at their figures eliminating postmenopausal women? Second, I am concerned about their recommendations that repeat Pap smears are poor methods of evaluating a class II smear when they sent their Pap smears to a state laboratory and I would like to know if they know anything about the laboratory. It is the same cytologist who is reading it? Are they board-certified? I really feel that that is a strong recommendation to make when it is not being sent to a single cytologist and one does not know who is reading the smear.

Unknown Speaker. I would like to ask Jones et al. how they identified the patient after they had taken the picture. I did not see a marker on the picture.

Unknown Speaker. I would like to ask Jones et al. whether they distinguished between dysplasia and colpocytologic changes caused by human papilloma virus. If this distinction was not made it is likely that they found a high number of abnormalities associated with normal Pap tests.

Dr. W. Siegfried Smith, Jr., Greensboro, North Carolina. I would like to ask Jones et al. if they performed a conization after a couple of negative colposcopies. What were the figures for false positive and false negative results?

Dr. Jones (Closing). The survey and 35 mm pictures were done predominantly by one of the faculty. It is even easier than working a 35 mm camera. Most physicians who do this have a nurse who takes the pictures.

What would happen if we eliminated the postmenopausal patients? It would make the surveys look much better. Quite frankly, most of the cases in which the transformation zone could not be seen were in postmenopausal patients. We have the same problem you do when the exocervix is entirely normal in the postmenopausal woman. It is not a technology that is applicable to everybody. I do not have any figures as to what happens if we remove the postmenopausal women; we took all patients as they came.

There may be some confusion as to who does Pap smears. All the initial Pap smears were done in local health departments and the patients were referred to us. Once the Pap smears arrived at our institution they were reviewed and read by the Department of Pathology at East Carolina University and specifically screened by three cytotechnologists and reviewed by two pathologists. We know who they are and we have a fair degree of confidence in the Pap smears that we obtained in our clinics.

How did we identify the patients? The numbers are small but on the bottom right-hand corner of the cervigrams there are patient identifier numbers, so there is no way of getting them confused.

We fought the game of what is dysplasia and what is condyloma before we started this study and, yes, if you include patients with koilocytotic atypia or condylomatous atypia and start calling them dysplasia your numbers go all off the wall. I think we have some fairly rigid definitions of what represents a viral alteration of the epithelium and what represents dysplasia. I feel a fair degree of confidence when I talk about dysplasia.

Those patients who had unsatisfactory colposcopy underwent conization. There were two such patients who underwent cold-knife conization; if we had an initial Pap smear discrepancy we simply went back and redid the colposcopy and in most instances that permitted us to resolve that discrepancy. We performed conizations on all patients who had cervical intraepithelial neoplasia II or cervical intraepithelial neoplasia III Pap smears in whom we could find no lesion. We did not perform conizations on those patients who repetatively could be found to have no histologic abnormalities if they had single cervical intraepithelial neoplasia I Pap smear that we just could never prove by aggressive trials.

Regional program for prevention of premature birth in northwestern North Carolina

Paul J. Meis, M.D., J. M. Ernest, M.D., Mary Lou Moore, R.N.C., Ph.D., Robert Michielutte, Ph.D., Penny C. Sharp, M.Ed., and Paul A. Buescher, Ph.D.

Winston-Salem and Raleigh, North Carolina

We present our experience with introducing and implementing a prematurity prevention program in a 20-county region in northwest North Carolina. The program is based on the risk assessment and educational model. In the first 25 months of results available, 17,370 births occurred in women enrolled in the project. During 1985, 42.5% of all births in the region occurred in the project. During the time span examined, a downward trend was seen for rates of low birth weight births in public and private patients and for very low birth weight births in private patients. During 1985, private births in the project were significantly less likely to be low birth weight or very low birth weight than private births to women not in the project. These results suggest that regional implementation of prematurity prevention programs is feasible and that such programs are particularly appropriate for private patients. (Am J Obstet Gynecol 1987;157:550-6.)

Key words: Premature birth prevention, regional perinatal care

Most southern states, including North Carolina, experience high rates of infant mortality.[1] The majority of these large numbers of infant deaths are due to premature or low birth weight (LBW) births. Although some progress has been made in reducing the rate of infant mortality in recent years in North Carolina, little reduction has occurred in the rates of LBW births,[2] as shown in Table I. Significant further reduction in infant deaths will be achievable only by reducing the number of LBW births.

Although general agreement exists about the need for reducing the rate of LBW births, there is not a clear consensus on the means to effect this reduction or with respect to the value of current prevention strategies. The utility of a program of risk assessment, patient and provider education, and life-style modification for patients at risk has been described by Papiernik et al.[3] and found to be effective by some authors[4] and ineffective by others.[5] The potential value of this approach suggests it merits further testing.

According to the general principles set forth by Papiernik et al., a prematurity prevention project in northwest North Carolina was initiated to determine: (1) if these prevention methods could be implemented on a regional basis involving a heterogeneous population of physicians, nurses, and pregnant women in a southern state and (2) if these methods could be shown to reduce the rate of LBW births in the population of patients compared over time or compared with the LBW rates of contemporary patients not involved in the project.

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Description of the program

The geographic site of the program, Perinatal Region 2, is a 20-county region in northwest North Carolina with a population of about 1,650,000 and approximately 20,000 births per year.

In meetings with physicians, nurses, county health directors, and others throughout this region, the program was explained and offered to health care providers. All of the county health department clinics and many private obstetricians and family physicians chose to use the program. Other physicians chose not to participate and pregnancy outcomes of their patients can be compared with those of patients involved in the program.

The elements of the program include: (1) risk assessment of all patients at the initial prenatal visit and again at 24 to 28 weeks, evaluated by means of a risk assessment tool derived from the work of previous investigators[6, 7]; (2) education for all patients about the signs and symptoms of premature labor and what to do if these occur; (3) education of all personnel in the health department clinic or private office concerning the importance of prompt evaluation of patients who

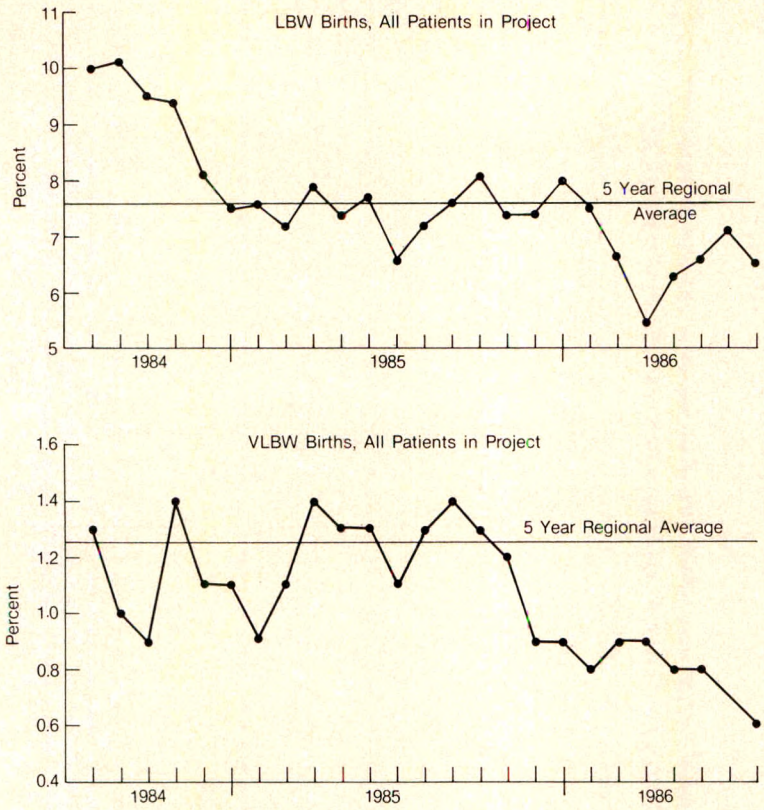


Fig. 1. Rates of LBW and VLBW births to all women in the project, shown as 3-month moving averages.

Table I. Percents of LBW births

	Year				
	1980	*1981*	*1982*	*1983*	*1984*
Northwest North Carolina					
501-1500 gm	1.25	1.24	1.20	1.18	1.28
501-2500 gm	7.24	7.39	7.73	7.28	7.78
North Carolina					
501-1500 gm	1.31	1.33	1.32	1.29	1.32
501-2500 gm	7.81	7.75	7.82	7.68	7.72

may be in premature labor; (4) intensive prenatal care for patients at risk for preterm births with weekly visits and cervical examinations after 24 weeks' gestation; (5) assessment of uterine activity of all patients with symptoms or signs of premature labor including appropriate use of tocolytic agents.

The program was explained, organized, and initiated in each clinic or private office by a personal visit of a nurse-educator employed by the program.

Methods

In evaluating the results of this program, all births of 501 to 2500 gm were classified as LBW and a subset of these births, 501 to 1500 gm, were defined as very low birth weight (VLBW). Births of <501 gm were not included as these births are variably reported as either live births or as spontaneous abortions.

Preliminary evaluation of the effectiveness of the project in reducing LBW births has been conducted by means of two methods. Rates of LBW and VLBW births occurring in women enrolled in the project are examined as trends from the first month of large numbers of births recorded, July 1984, to the most recent month of available data, July 1986. These data are examined by provider class: public patients from county health department prenatal clinics and private patients from private practitioners.

Second, data obtained from birth certificates for all

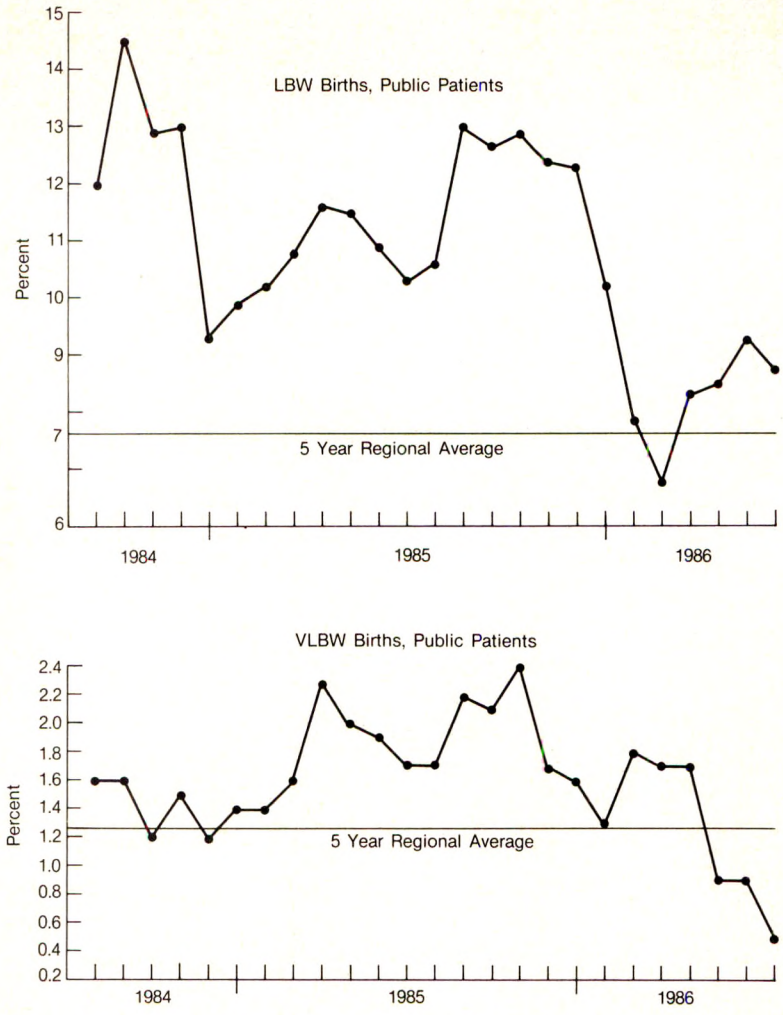


Fig. 2. Rates of LBW and VLBW births to women in the project receiving care through health department clinics.

births occurring in Region 2 in 1985 were used to compare the results of births to women in the project with births to women out of the project for 1985. Comparisons in rates of LBW and VLBW birth were made with the use of χ^2 analysis with the criterion for statistical significance at ≤ 0.05.

Results

During the time span examined, 17,370 births occurred in women enrolled in the project. For the calendar year 1985, 42.5% of all births in the region were in the project. The rates of LBW and VLBW births that occurred are shown in Fig. 1 and expressed as 3-month moving averages in order to smooth month-to-month variability. Included in Figs. 1 through 3 are historical rates of LBW (7.48%) and VLBW (1.23%) births for Region 2 for the 5 years 1980 to 1984. A downward trend is seen for rates of LBW and VLBW births, with recent cohorts of LBW and VLBW birth rates well below historical rates for the region.

Fig. 2 shows similar data for public births, which totaled 5123 (29.5% of total births in the project). Greater fluctuation is seen in rates of LBW and VLBW births, which can be explained in part by the smaller number of births in each cohort. Although rates of LBW and VLBW births are high, a downward trend is seen in the rate of LBW births, and recent cohorts show a marked improvement in rates of VLBW birth.

Fig. 3 shows results of births in private patients, whose 12,247 births constituted 70.5% of total births in the project. A downward trend is seen in rates of LBW births, and rates of VLBW births are particularly low for the last 12 months of births.

Comparisons of rates of LBW and VLBW births of private patients enrolled in the project with private patients not enrolled in the project are shown in Table II and Fig. 4. Patients receiving care through county health department clinics and patients who received no prenatal care were excluded from these data. Since all of the public health departments were participating in

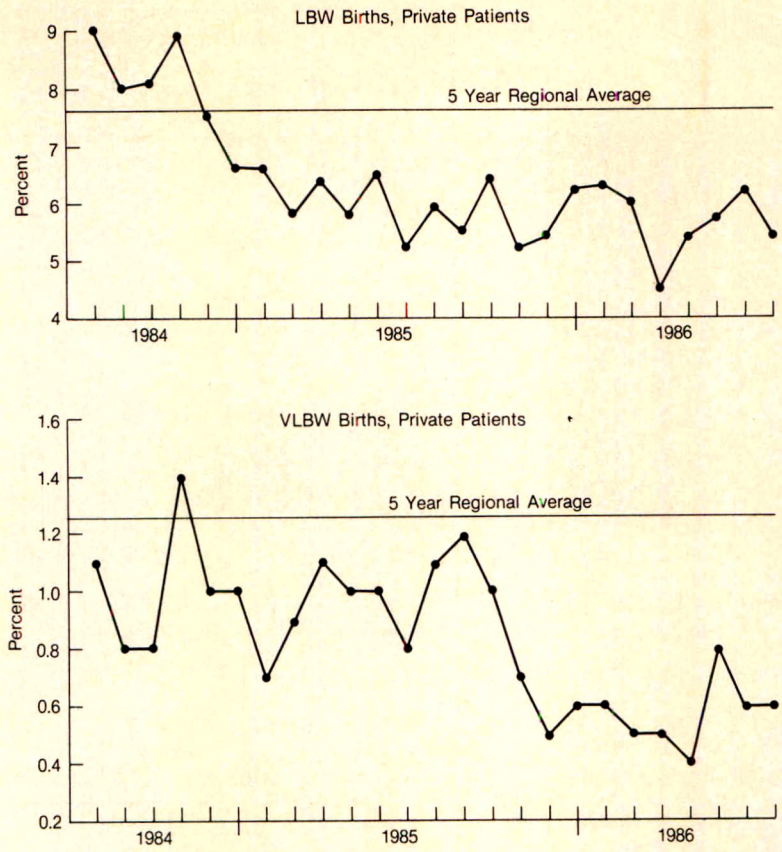


Fig. 3. Rates of LBW and VLBW births to women in the project receiving care from private physicians.

Table II. Births in private patients who received prenatal care in northwest North Carolina in 1985

	Not in project		*In project*			
	n	%	*n*	%	χ^2	*p*
Total	11,850		6,477			
501-1500 gm	151	1.27	57	0.88	5.80	0.012
501-2500 gm	870	7.34	380	5.87	14.33	0.0001

the project, a comparison of public patients in and out of the project is not possible. Rates of LBW and VLBW births were significantly lower in private patients enrolled in the project compared, by means of χ^2 analysis, with private patients not enrolled in the project. The demographic characteristics of private patients in and out of the project were examined with the use of information obtained from birth certificate data through the State Center for Health Statistics. It was possible to match 88% of 1985 births in the project with birth certificates. Some demographic characteristics are shown in Table III. The group of patients not in the project had slightly higher rates of some high-risk parameters. A more detailed description of the demographic characteristics and relationship to outcome measurements is considered elsewhere (Buescher PA, Meis PJ, Ernest JM, et al. A comparison of women in and out of a prematurity prevention project in a North Carolina perinatal care region. Submitted for publication).

Cost savings. The direct and indirect financial costs of VLBW birth (<1500 gm) are considerable. In the intensive care nursery at our hospital, hospital and physician bills for the care of these infants average $29,900. Costs in other areas of the United States are much higher. Approximately 15% of these infants will be expected to suffer from cerebral palsy. The average additional expense of raising a child with cerebral palsy to age 18 is $95,083.[9] Thus, as shown in Table IV, the average direct financial cost of each VLBW birth in northwest North Carolina is $44,162.

If the 8952 parturients enrolled in the program in

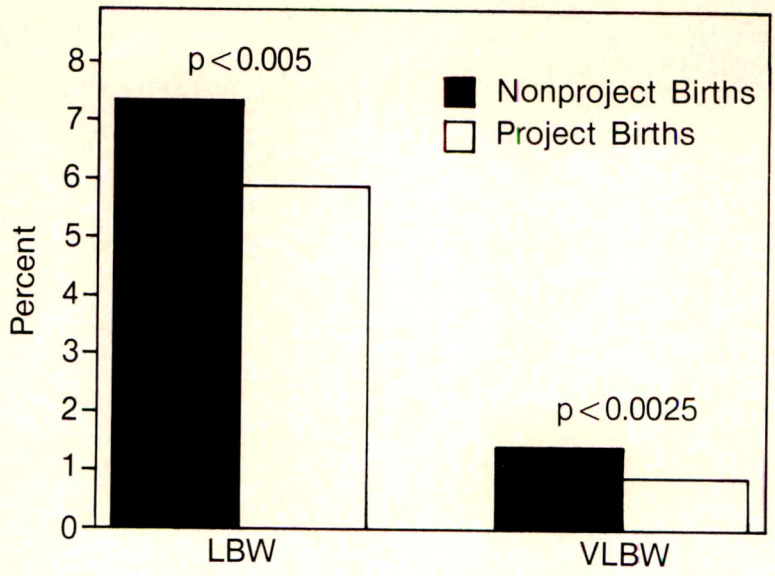


Fig. 4. Comparison of rates of LBW and VLBW births to women cared for by private physicians using the project with those to women cared for by physicians not using the project.

Table III. Demographic characteristics of private births in northwest North Carolina in 1985

	Not in project (%)	*In project (%)*
Nonwhite	19.1	14.6
Unmarried	17.2	12.2
Under age 18	5.4	4.5
Education <12 yr	23.0	20.0
Previous fetal or live-born death	16.7	17.6
Complications of pregnancy	13.0	12.8
Medicaid	8.1	5.0
WIC Program	12.7	9.5

Table IV. Average medical expenses of VLBW infants

Cost of raising child with cerebral palsy	$95,083.00
Occurrence rate of cerebral palsy	× 15%
	= $14,262.45
Mean neonatal intensive care unit expense	+29,900.00
Direct expense of <1500 gm birth	= $44,162.45

the most recent 12-month period had experienced VLBW birth at the average rate for all women in Region 2 (1.31% for 1985), an additional 23 VLBW infants would have been delivered.

This reduction in VLBW births represents a cost savings of 23 × $44,162 or $1,015,736. The direct costs of supporting the prematurity prevention program during this time period was $90,123, which represents a cost savings of $11.27 for each dollar spent on the program.

Comment

This examination of the first 2 years of births in a prematurity prevention program shows promising overall trends in reductions of LBW and VLBW births in a group of 17,370 births. Rates of LBW and VLBW births for the first calendar year of the project, 1985, also are lower than those in a contemporary group of private patients not participating in the program.

The design of this study, emphasizing widespread use throughout a region, does not permit a truly controlled study. In order to evaluate the effectiveness of such a program, an extended time span may be required to show continued improvement in rates of LBW and VLBW births. Papiernik et al.,[3] in their pioneer work in prematurity prevention, required 4 years before demonstrating effective results. Cole[10] has described the problems of maintaining effective prematurity prevention programs in the United States, where funding for programs is often for short time periods and results are expected to be demonstrated in 3 years or less.

Reductions in LBW births are most striking among private patients and among VLBW births. This observation is consistent with our previous findings that a greater percentage of LBW births in private patients is

related to idiopathic premature labor, rather than medical problems, premature rupture of the membranes, or impaired fetal growth.[11] Similarly, VLBW births were more often related to idiopathic premature labor than LBW births. Thus the apparent success or lack of success of prematurity prevention programs is likely to be affected strongly by the characteristics of the patients included in the program. Prematurity prevention projects of the type described here that focus solely on urban, inner city populations may have less chance for demonstrating success than those that are directed to other patient populations.

The data suggest that this project may be most successful in reducing the rates of VLBW infants. It is important to emphasize that since these births make up only 1% to 2% of all births, a large cohort of births must be examined to identify a significant reduction. Several previous studies have had relatively small cohorts of parturients, making examination of reductions in rates of the VLBW category difficult.[4, 5] Nonetheless, births in this category are extremely important because of the high rates of morbidity and infant death and the high financial and social costs associated with these births. Even modest reductions in the rates of VLBW births can make prevention programs extremely cost effective.

In summary, this project demonstrates that current prematurity prevention strategies can be implemented for a wide segment of private and public patients on a regional basis. The program has been received enthusiastically by many private obstetricians and family physicians. These preliminary results suggest this model may be particularly effective in a private practice setting. Continued examination of these trends over a multiyear time span will be needed to confirm these results.

Further reductions in premature births could be accomplished by: (1) wider promotion and adoption of this risk assessment, educational, and intervention model; (2) efforts to ensure that prenatal care is available to all pregnant women regardless of income; and (3) further investigation about the causes of premature birth and, in particular, premature rupture of the fetal membranes.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. WILLIAM N. P. HERBERT, Chapel Hill, North Carolina (Official Guest). Dr. Meis and colleagues have reported their experience with a project aimed at decreasing premature births over a wide geographic area of our state that encompasses almost 20,000 births per year. With the use of a combination of patient education of the signs and symptoms of preterm labor, prenatal risk assessment with close surveillance of high-risk gravid women, and prompt evaluation of all patients with excessive uterine activity, they found a downward trend in the rates of LBW and VLBW infants for both private and public births. In addition, they presented the substantial financial savings calculated from having fewer preterm infants delivered.

As noted by Dr. Meis, others who have addressed this issue have reported conflicting results. Papiernik et al.,[1] using a multifaceted approach, found a significant reduction in preterm births, most impressive in the earliest gestational age groups and in the lowest social groups. Interestingly, patients in the highest risk group did not show a reduction in the rate of preterm delivery. Herron et al.[2] have also noted an improved preterm delivery rate with such a program in a supposedly middle-class population in San Francisco, California. However, Main et al.,[3] utilizing an "inner city" population in Philadelphia, Pennsylvania, reported no benefit of a preventive program of this type. In reviewing this manuscript and pertinent literature to attempt to explain these different results, several issues emerge that concern the questions asked in such studies and the answers provided in them. I would like to ask Dr. Meis to comment on these issues, using his experience in this area.

First, in looking at preterm birth prevention projects, are the proper questions being asked? These programs are directed toward early detection of preterm labor,

presuming that prompt intervention can forestall delivery. However, as it has been shown that preterm labor itself is responsible for only about 30% of preterm births,[4] the real benefactors of these programs, that is, patients with idiopathic preterm labor, may be lost in the much larger group of patients with premature rupture of the membranes or one of a host of maternal-fetal problems associated with or necessitating early delivery. Outcome data on the entire population may mask the benefit in this select group. It seems that only by focusing on patients with preterm labor of unknown cause can we really evaluate a preterm birth prevention program as described.

Second, are the appropriate answers being provided in these studies? The standard practice of using either delivery before 37 weeks' gestation or an arbitrary LBW definition of <2500 gm (or <1500 gm for VLBW infants) provokes a yes/no response in data outcome tabulation that can interfere with meaningful analysis. Would not a shift upward in the mean birth weight—say, from 1500 to 1900 gm—or a shift upward in mean gestational age—say, from 30 to 33 weeks—be very important? Furthermore, we must remember that, as shown by Goldenberg et al.,[5] a seemingly small prolongation of gestation, even 1 week, can have a significant effect on ultimte survival of these infants. Dr. Meis has shown us that the savings in medical care costs can also be substantial. Thus we cannot forget the strong impact of even slight shifts as we analyze data from these studies.

Certainly, preterm births involve multiple factors, among them obstetric history and other historical events, early detection of uterine activity, and the state of the cervix. The ideal study with a population large enough to be meaningful with a control population worthy of comparison would be extremely difficult to design. Moreover, preterm birth prevention programs need time to show their real colors. Papiernik's program required 4 years to show an effect, for instance, and it would be extremely unfortunate to have programs lose their funding base and support because of this so-called type III error, that is, erroneous evaluation of a program that has not been adequately implemented.[6]

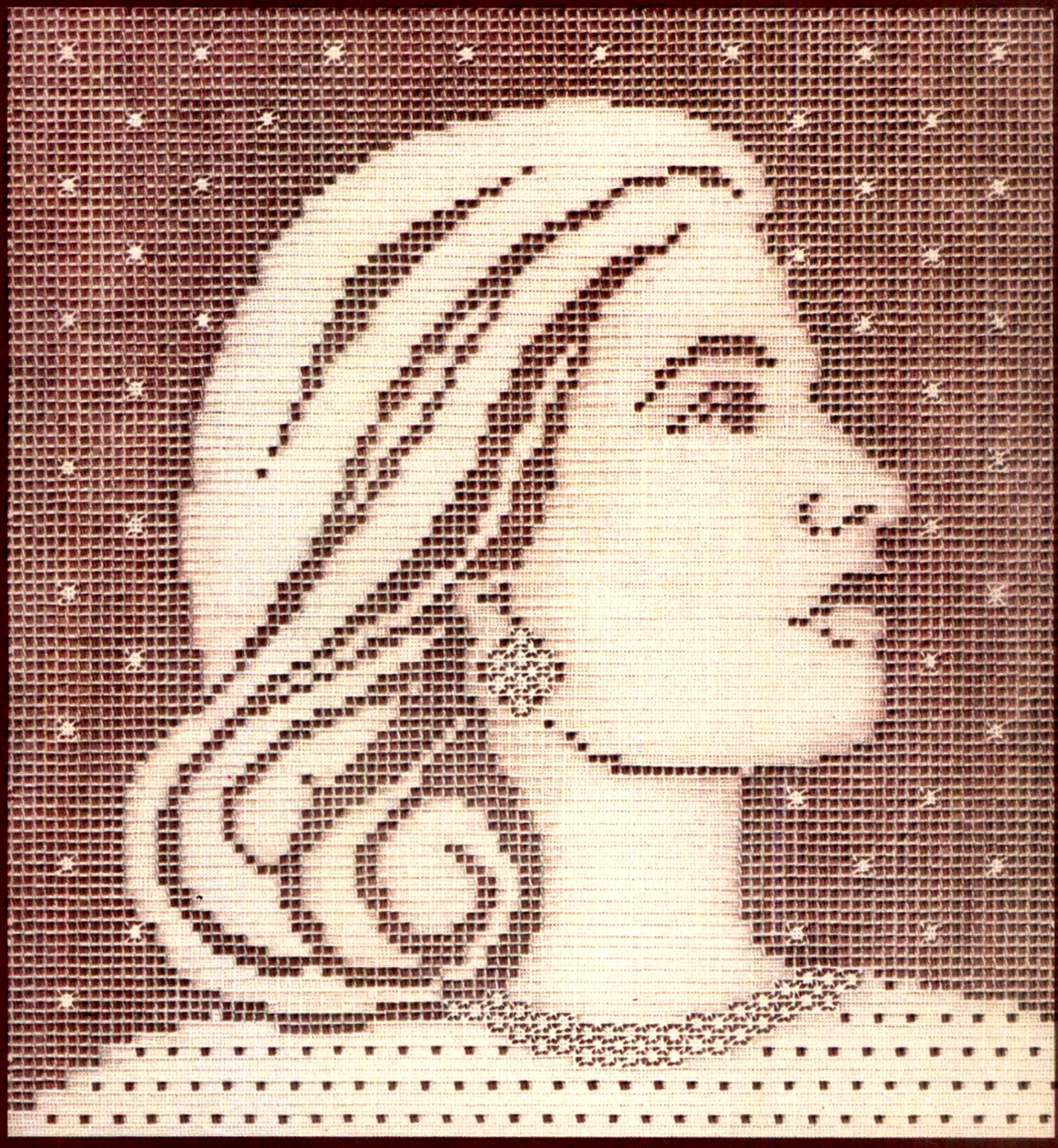
As complex as preterm labor appears, however, the enormous magnitude of the mortality and morbidity of preterm births should fuel our efforts to reduce this complication that we so often see. Dr. Meis and his coworkers are tackling this issue and their continued work will help to further our knowledge of this important subject.

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Dr. Meis (Closing). Dr. Herbert's comments are pertinent and thoughtful and suggest several additional methods for future analysis of results. I would emphasize some of the problems in evaluating results of this or similar programs to reduce rates of preterm birth. These problems include the time needed to obtain results and the importance of evaluation of the VLBW category. The time required to obtain results is fairly substantial. Approximately 1 year is needed to recruit the help of health care providers to initiate the program. A second year will elapse from the time of patient enrollment at the first prenatal visit until results of their pregnancy outcome are available. Frequently some time will elapse before improvement in the results of these pregnancy outcomes is noted. As Dr. Herbert mentioned, Papiernik's study took 4 years before showing improved results. Most public health projects are funded on a 3-year basis and funding agencies will expect results within 2 years before considering an extension of the funding. The VLBW group, as we have indicated, is an important group to consider because of the especially high morbidity and mortality rates and the very high financial costs involved. In addition, concern has been expressed that prematurity prevention strategies may simply shift the births from a previable group up into this VLBW group. The need for large cohorts of patients and the difficulty of obtaining these cohorts in a randomized prospective trial in a single geographic area suggests that alternative experimental designs are needed.

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CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only OCs have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. (The rates given for Pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only OCs are used), and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving OCs, since the drugs are more effective in preventing intrauterine than ectopic pregnancies.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination being performed.

2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See patient package labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs:

a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thryoid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis, Mutagenesis, Impairment of Fertility—See "Warnings" section #3, 4 and 5 for information on carcinogenesis, mutagenesis and impairment of fertility.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings." Because of the potential for adverse reactions in nursing infants from OC tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis; pulmonary embolism; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; benign hepatomas; congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria; hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Levlen must be taken exactly as directed and at intervals not exceeding 24 hours.

Any time the patient misses 1 or 2 light-orange tablets, she should also use another method of contraception until she has taken a light-orange tablet daily for 7 consecutive days.

For full details on dosage and administration, see prescribing information in package insert.



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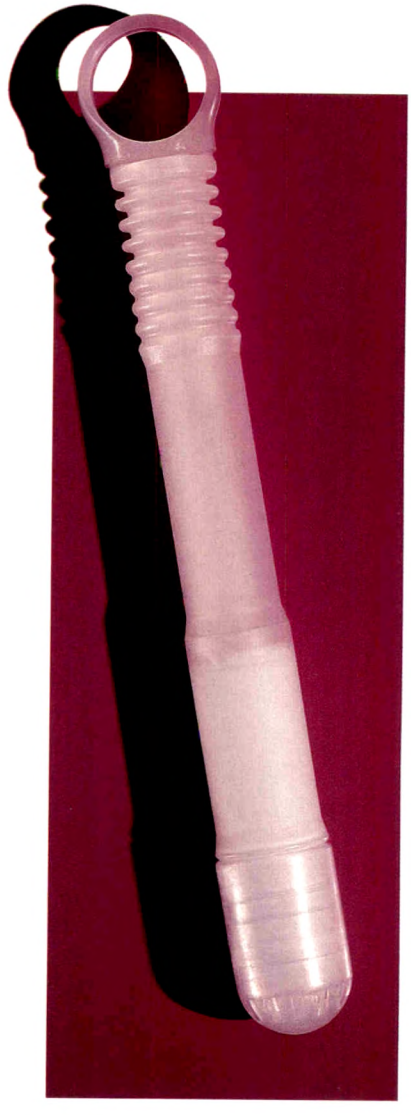
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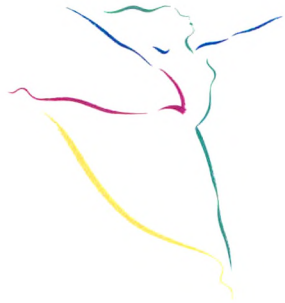
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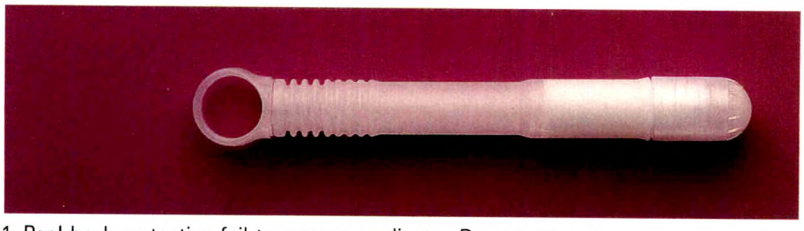
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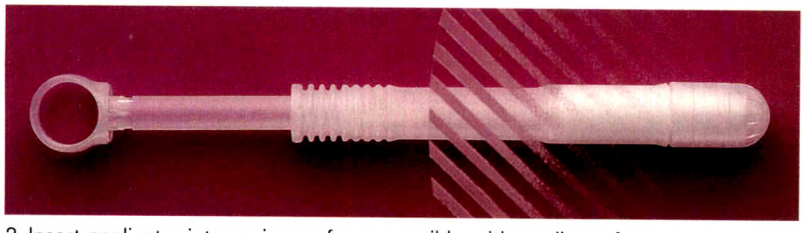
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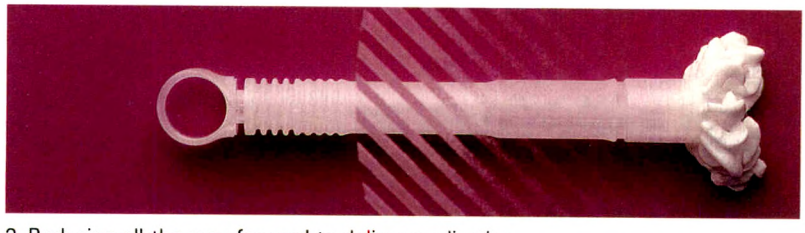
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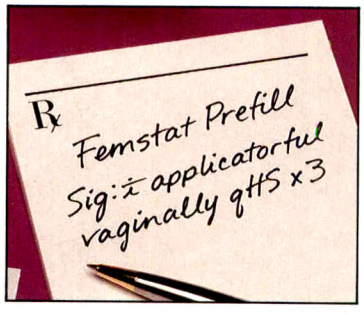


2. Insert applicator into vagina as far as possible without discomfort. Pull back on ring.



3. Push ring all the way forward to deliver medication. Withdraw applicator and discard.





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BRIEF SUMMARY

INDICATIONS:
FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS:
FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS:
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Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

February 1987

Careful surgical technique can reduce infectious morbidity after cesarean section

James B. Lyon, M.D., and A. Cullen Richardson, M.D.
Atlanta, Georgia

A total of 635 consecutive cesarean sections performed in private practices during the past 20 years are reviewed. In the course of this 20-year period, we have tried to alter gradually our surgical technique used for this procedure to conform to the principles of surgery developed by Halsted. The study is divided into three periods of time to examine the results of changes in technique. With the use of the standard definition of febrile morbidity (temperature of ≥100.4° F during any two 24-hour periods after the first postoperative 24 hours) the postoperative morbidity rate was 15% during 1967 to 1973, 5.4% during 1974 to 1979, and 0.7% during the period 1980 through 1986. Prophylactic antibiotics were used with decreasing frequency. It was clear that the reduction in morbidity was the result of the modifications in surgical technique. (Am J Obstet Gynecol 1987;157:557-62.)

Key words: Cesarean section, febrile morbidity, surgical technique, suture material

Infectious morbidity after cesarean section continues to attract much attention and study. A recent bibliographic search through the National Library of Medicine found more than 600 articles published during the past 15 years on the use of antibiotics either prophylactically or therapeutically to reduce infectious morbidity after cesarean section. No article could be found that addressed directly the effect of surgical technique on infectious morbidity.

Almost 100 years ago Dr. Wm. S. Halsted began teaching the principles of meticulous surgery. The gentleness in the handling of tissue, careful hemostasis, asepsis, and the precise approximation of layers are just as important today as they were then. Without these principles successful major surgery in the 1890s was impossible. Without these principles today, major operations such as cesarean section are still fraught with high rates of infectious complications. During the past 20 years an attempt has been made to translate the principles of meticulous surgical technique into practice when cesarean section is performed. A review of this 20-year experience forms the basis of this report.

Material

A total of 635 consecutive cesarean sections that we performed during the period from Jan. 1, 1967, to Dec. 31, 1986 (20 years), were reviewed. These sections were done as incidents in the management of 4040 private obstetric deliveries. The demographic characteristics of the practice population were fairly constant throughout the period and consisted of 70% white and 30% black, predominantly middle-class patients. The only demographic characteristic that changed during this 20-year period was patient age. The average age of the patient undergoing cesarean section was slightly more than 25 years in 1967 and was almost 30 in 1986.

Our cesarean section rate changed markedly during this 20-year period. It was at slightly more than 5% in 1967 and grew to almost 20% in 1986.

During the early years of the study, 68% of the cesarean sections were primary, and only 32% were repeat operations. With the increasing cesarean section rate, repeat operations have increased and now constitute almost 50%. Encouragement of vaginal delivery after cesarean section has been done only in the past 2 years and has had little effect as yet on the rate.

As this study was designed to determine to what extent surgical technique affects postoperative morbidity, particular attention was given to fine points of operative routines.

Certain practices were essentially constant throughout the 20-year period. The traditional methods of careful asepsis to include careful skin preparation before operation were followed throughout. Almost all operations were done through a Pfannenstiel incision. The uterine incision was transverse in more than 90%; vertical incisions were reserved for patients in whom the lower uterine segment was not well formed. Regional anesthesia was used in more than 90% of the patients in all three groups. The only difference was that in group 1 patients this was usually spinal anes-

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Table I. Total number of deliveries and rate of cesarean section

	Group 1	*Group 2*	*Group 3*
Total deliveries (n)	1925	1625	1490
Cesarean sections (n)	135	203	297
Cesarean section rate (%)	7.0	12.5	19.9

thesia and in group 3 it was almost uniformly epidural anesthesia. Spinal and epidural anesthesia were used about equally in group 2.

Method

It was about 20 years ago when we began a diligent attempt to modify our operative technique in a large number of little ways. Every aspect of surgical technique was questioned, for example, the choice of suture material and needles for uterine and abdominal closure, the desirability of the use of transurethral catheters in the bladder and laparotomy pads in the abdomen, every minor aspect of tissue handling, and many other quite small to minute points of technique that might affect tissue reaction. Some changes were tried and abandoned. Those that were seen to be beneficial were retained. This has been an ongoing slow and continuing evolution. The changes that were made tended to evolve rather than to be invoked at a specific time.

However, in analyzing the results, it seemed appropriate to divide the 20 years into three time periods: 1967 through 1973 (group 1), 1974 through 1979 (group 2), and 1980 through 1986 (group 3). The first dividing line at the end of 1973 was chosen to conform to the beginning routine use of synthetic absorbable polyglycolic acid suture instead of catgut and silk, respectively, for closure of the uterine and abdominal incisions. The second division at the end of 1979 was chosen to conform to the change in the hospital in which we conducted our practice. All of our deliveries and cesarean sections from 1967 to 1979 were performed in a teaching hospital with residents as assistants. In late 1979, because of changes in the demographic features of our city, we moved our office and in turn we changed our hospital of practice to a private, nonteaching hospital with an obstetric service of approximately the same size. The anesthesia staff, operating room facilities, and level of asepsis appeared the same in the two hospitals. However, as there are no residents, all operations in the past 7 years have been done with the assistance of another fully trained practicing obstetrician-gynecologist. (Meticulous operation is more easily performed with experienced help.)

The size of each group with the cesarean section rate for each period is shown in Table I.

Twenty patients underwent planned cesarean hysterectomies at the time of repeat operation and four had unplanned emergency hysterectomies. They were equally distributed among the groups.

Comment

The rationale underlying our changes in technique was based on the concept that although bacteria are present in all wounds, their multiplication and growth require a culture medium of killed or damaged tissue with accompanying exudate and/or blood. This indicates that every possible attempt be made to reduce tissue damage. Our experience with the use of this approach for abdominal hysterectomy has been reported previously.[1, 2]

To develop a consciousness about tissue trauma, one must think at the level of the individual cell and rebel at the idea of killing or damaging even one cell unnecessarily. It is difficult to define objectively what to some might appear to be minor differences. A few examples with our reasons for the changes follow. At the beginning of the series, subcutaneous bleeders were clamped hurriedly with standard hemostats; today they are carefully clamped with mosquito hemostats. The smaller the number of fat cells destroyed by the hemostat, the less the amount of free oil and cellular debris left in the incision. In 1967, we routinely placed laparotomy pads into the paracolic gutters; today this is never done. Laparotomy pads can abrade serosal surfaces. In the 1960s, we always used an indwelling transurethral catheter in the bladder during operation, leaving it in place for 24 hours after operation; today the indwelling transurethral catheter is never used before, during, or after operation. A considerable percentage of urinary tract infection can be eliminated by omitting transurethral catheters. Until the late 1970s, scissors were frequently used to cut tissue; today all tissue is cut with a sharp knife. Scissors invariably pinch tissue apart and cause more trauma than a sharp knife.

Most of the many changes were similarly small, but all were intended to reduce the number of killed or damaged cells left in the patient as she went to the recovery room.

There have been several changes in suture material and needles used. Before the advent of polyglycolic acid suture in the early 1970s, the uterus was routinely closed with chromic catgut and generally the abdominal wall was closed with silk. Today polyglycolic acid sutures, of much smaller sizes and placed with much smaller needles (3-0 Dexon on a T-16 needle), are used for both. At the beginning of the period, interrupted 2-0 and 3-0 silk sutures were used to close the skin. Currently this is done with an intradermal suture of 6-0 polyglycolic acid suture (6-0 Dexon on a CE-2 needle). The synthetic absorbable sutures excite much less

tissue reaction than do catgut and silk. The larger the needle passed through tissue, the greater the trauma done to that tissue; thus the needle size has been decreased several times.

Another major change has been in the placement of sutures as close as possible to the edge of the layer being sewn. This results in a narrower wedge of and volumetrically a smaller amount of tissue within the suture line. Tissue within a tied suture has some diminution in blood supply and tissue perfusion.

Also, during the past 6 years, in closing the uterus no sutures are passed into the endometrial cavity. As long as a suture remains traversing the endometrial surface, the endometrial lining cannot reseal and the suture can have a wicking action, allowing the bacterial flora of the endometrial cavity constant upward access to the myometrium.

Prophylactic antibiotics have been used only in patients having primary operations and here with decreasing frequency as they seem to be unjustifiable from a cost/benefit analysis unless there appears to be infection present at the time of operation. There has been no hesitancy to use them postoperatively if there is any hint of infection as manifested by anything from a slight temperature elevation to faint erythema around the incision. Nevertheless, 29% of the patients in group 1 received some antibiotics, 25% in group 2, and 11% in group 3. In group 1, the antibiotic of choice was injectable penicillin with or without streptomycin. In group 2, the majority received oral ampicillin or injectable penicillin with or without streptomycin. In group 3, 29 of the 33 patients given antibiotics received oral ampicillin or injectable penicillin, two patients received cefoxitin, one received cefazolin, and one, oral erythromycin. In only 10 (3.4% of the Group 3 patients) were the antibiotics begun prophylactically at the time of operation.

No attempt was made to culture the uterine contents unless infection was suspected at the time.

Results

There has been a progressive reduction in postoperative morbidity. Table II shows the febrile morbidity, defined in this study as temperature of ≥100.4° F (38° C) in any two 24-hour periods after the first postoperative day. Also shown is the length of postoperative hospital stay.

There have been only two patients with febrile morbidity among the 297 group 3 patients: One had severe pregnancy-induced hypertension with convulsions (eclampsia) and the other had a placenta accreta, in which the placenta required removal by sharp dissection with some attendant myometrial damage and resultant endomyometritis. The patient with eclampsia did not receive prophylactic antibiotics. The patient with placenta accreta received cefoxitin at the time of operation. Neither had any temperature recording above 101.8° F, and both recovered promptly with postoperative hospital stays of 7 and 6 days, respectively.

Table II. Febrile morbidity and length of stay after cesarean section

	Febrile morbidity (%)	*Postoperative stay (days)*
Group 1	15.0	4.9
Group 2	5.4	3.8
Group 3	0.7	3.5

The blood transfusion rates were between 4% and 5% throughout the 20 years, with no significant difference between the three groups of patients. The cesarean hysterectomies had no effect on morbidity rates but did influence the rate of transfusion as more than half of them required some blood replacement.

All patients in the series have been seen at least once in the office for postoperative follow-up at 4 to 6 weeks post partum (most have been followed up for years). No pelvic abscesses were detected in any patient in the entire series. There were three incisional hematomas requiring drainage in the office among the group 3 patients and two in group 2. (No hematomas were recorded in group 1.) None of these patients was febrile. There were no incisional abscesses or disruptions. Only one patient (from group 2) became febrile after discharge and required readmission to the hospital. She had a small broad ligament hematoma. She responded promptly to antibiotics without drainage.

All of the morbidity in groups 2 and 3 occurred after primary cesarean section. There has been no morbidity in more than 200 repeat operations and none of these patients received prophylactic antibiotics. No consistent relationship was found between morbidity and the number of vaginal examinations, internal fetal monitoring, length of time of ruptured membranes before cesarean section, or type of anesthesia.

There were no maternal deaths. There was one serious complication in this series in a group 2 patient. The cesarean section was done in this patient because of failure to progress in labor with twins. The operation was begun with the patient under epidural anesthesia with the delivery of two healthy infants. During the closure of the abdomen, the anesthesia in the abdominal wall began to fade and the anesthesiologist elected to switch to light general anesthesia. The patient was given pentothal sodium and a bolus dose of succinylcholine. Almost immediately the patient had tonic contractions of all muscles and shortly thereafter began to manifest signs of malignant hyperthermia. In spite of very aggressive treatment, the body temperature rose

above 108° F. She sustained severe central nervous system damage.

It might appear that with the large increase in cesarean section rate there has been only a dilution of the so-called inevitable sepsis in this group of patients. However, when one divides the number of cesarean operations associated with sepsis by the number of deliveries during each period, it is apparent that 0.88% of the obstetric patients in group 1 were morbid as a result of cesarean section; in group 2, 0.68% were morbid as a result of cesarean section; in group 3, 0.13% experienced morbidity as a result of cesarean section.

The only negative result of this approach was, as might be suspected, a slight prolongation in operating time. As one becomes more meticulous, additional time is required to perform the operation. At present the usual operating time is between 50 and 75 minutes. With larger needles and sutures and big bites of tissue, the operating time was much shorter.

Comment

Danforth,[3] in an excellent review article in 1985, gave the prevailing morbidity rates for cesarean section as 50% to 65% without prophylactic antibiotics and 15% to 26% with antibiotics. Duff and Kopelman[4] in a similar review article reported febrile morbidity rates of 44% without and 20% with prophylactic antibiotics.

There are difficulties in comparing an all-private patient study with studies of large numbers of indigent patients. Danforth in his review cited two Swedish studies[5, 6] that he felt might conform to the clientele of the average U.S. obstetrician in private practice. In these two series, prophylactic antibiotics were not used, and their rates of postoperative infection were 13.3% and 20%, respectively. Anstey et al.[7] in an all private patient study at St. John's Mercy Hospital in St. Louis, Missouri, reported a morbidity rate in primary cesarean sections of 23% without prophylactic antibiotics.

In one of the few recent articles that did not advocate prophylactic antibiotics, Iffy et al.[8] were able to show that with traditional preventive measures such as careful aseptic technique the infection rate after cesarean section was gradually reduced from more than 80% to 16.2%, without prophylactic antibiotics. Throughout the period of our study, we have tried at all times to follow the principles advocated by Iffy.

Ledger[9] reported the incidence of postpartum endometritis after abdominal delivery as being 10 times greater than that after vaginal delivery. Presumably the bacterial flora is similar in the two groups. The only difference is the incisions in the abdominal wall and uterus. With meticulous handling of these incisions, the ratio theoretically could be 1 to 1, not 10 to 1.

In the present study, the progressive decrease in febrile morbidity has been a significant one. The decrease in this series of patients is largely attributed to the changes in surgical technique–not better antibiotic prophylaxis, as antibiotics were used with decreasing frequency. The morbidity reduction from group 1 to group 2 seemed to be principally due to the change from catgut to polyglycolic acid suture. Refinements in tissue handling and suture placement and smaller suture and needle size seemed to be responsible for the additional improvement between groups 2 and 3.

In association with the decreasing morbidity, the patients were observed to have a generally smoother postoperative course with a resultant decrease in the postoperative hospital stay. In the last 297 (group 3) patients, the range in postoperative stay was 2 to 7 days; the average was 3.5 days.

To achieve the 0.7% morbidity rates reported in the last 297 cesarean sections, we pursued no new or special program. Nothing was done other than an attempt to be constantly aware of avoiding tissue trauma in every minute way possible. It was apparent to us that these little things made a difference in how well the patient fared after operation. The development of a consciousness regarding tissue trauma was a gradual evolution over the 20 years. It is our intention to continue to refine further this approach with the hope that we will be able to maintain morbidity rates of under 1%.

This study supports Halsted's century-old teachings that undamaged tissue can withstand very significant bacterial insult. Apuzzio and Iffy,[10] in an excellent book chapter on perinatal infection control, remarked after discussing Semmelweis' work: "Contemporary physicians are trained to believe that the progress of medicine is so rapid that research conducted a few decades ago is obsolete. . . . If infection control is to be effective and meaningful, any control measure must be proven to be unnecessary before it is abandoned." The same would apply to Halsted's teachings regarding surgical technique.

All wounds are contaminated—the cesarean section may be more so, yet undamaged tissue can withstand most of this contamination.

As indicated, the meticulous approach requires additional operating time, but as this study indicates the time is well spent.

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Discussion

DR. ROBERT M. WEIN, Greensboro, North Carolina (Official Guest). This report is an introspective evaluation of one group's 20-year experience with cesarean section and its associated febrile morbidity. The principles discussed should be widely held in all surgical procedures. Minimal tissue trauma, proper suture choice, good hemostasis, and asepsis do result in less febrile morbidity, fewer complications, quicker postoperative recovery, and shorter hospital stays, as Lyon et al. have shown.

For the purposes of comparison, to allow discussion, and to look at febrile morbidity in a slightly different perspective, all cesarean sections done at Moses Cone Hospital in Greensboro, North Carolina, between October 1, 1985, and September 30, 1986, were reviewed. During this 12-month time period, 943 cesarean sections were done: 80% were done on private patients by one of 24 attending obstetricians, and 20% were performed in teaching service patients where a resident was often assisted by an attending doctor. Overall, 69% of the cesarean sections were primary and 31% were repeat operations. According to the standard definition of febrile morbidity, the febrile morbidity rate was 2.6% on the private service and 9.7% on the teaching service. The total combined febrile morbidity rate was 4.0%. This corresponds very favorably to previously published figures, although it does not reach the 0.7% rate of Lyon et al. in the last 7 years of his 20-year review.

There are some other apparent differences in Dr. Lyon's group and our group. In our group, prophylactic antibiotics were used 81% of the time and usually consisted of one to three doses of a first-generation cephalosporin begun immediately after the umbilical cord was clamped. Objective evaluation suggests underuse by some of our obstetricians and overuse by others. In a comparison of individual practices, using prophylactic antibiotics 100% of the time did not result in the lowest individual febrile morbidity rates. On the other hand, 29% of our febrile morbidity occurred in the 19% of patients that did not receive prophylactic antibiotics. The use of antibiotic prophylaxis for women in labor requiring cesarean section may be appropriate.

Lyon et al. stressed the decreasing frequency of use of prophylactic antibiotics in his series. Certainly one cannot argue with their results without the use of prophylactic antibiotics. In their group 3, only 11% (33 of 297) received antibiotics; in 21 they were not given prophylactically but rather in response to "any hint of infection," and these 7% did not go on to satisfy the standard definition of febrile morbidity. Indeed the use of antibiotics can alter the febrile morbidity rate, which by definition requires a temperature >100.4° F occurring in two separate 24-hour periods after the first 24 postoperative hours. In fact, in our series, endometritis was diagnosed in 11 patients who once they were begun on a therapeutic regimen of antibiotics responded so well that they did not fit the standard definition of febrile morbidity. It may be more accurate to compare actual clinical situations such as endometritis, wound infection, or pelvis abscess rather than to use a strict definition depending only on temperature.

Lyon et al. emphasized the use of polyglycolic acid suture instead of catgut in closure of the uterus and abdomen to effectively lower postoperative febrile morbidity. In addition, attention to minimal tissue trauma by use of smaller gauge suture and smaller needles was discussed. The mechanics of polyglycolic acid suture breakdown in tissues by hydrolysis as compared with phagocytosis in catgut suture breakdown clearly establishes polyglycolic suture as less reactive. Interestingly, in our series, 21 of 26 (81%) patients developed postoperative endometritis after uterine closure with catgut suture. This is probably significant as 66% of our total group had uterine closure with catgut. This would support Dr. Lyon's thesis.

All of our patients undergoing cesarean section had a transurethral Foley catheter placed before operation, usually as part of the surgical preparation, and these catheters were removed on the first postoperative day. There were only seven urinary tract infections in the 943 patients and this low rate is probably related to our 81% prophylactic antibiotic usage. This is one area deserving of more comment by Dr. Lyon, in that he stated that an indwelling, transurethral catheter was never used before, during, or after operation. With the large amount of intravenous fluids given preoperatively and intraoperatively, especially when regional anesthesia is used, with procedures lasting between 50 and 70 minutes, and considering patient immobility after operation, it is difficult to imagine not using a catheter to keep the bladder decompressed during surgery to avoid bladder injury, to allow a true low transverse cesarean section to be done, and to aid in drainage in the immediate postoperative period.

Operative time at our hospital averaged 40 minutes. This ranged from an average of 25 minutes to 56 minutes for individual attending physicians and did not correlate with specific rates of febrile morbidity.

Our postoperative hospital stay averaged 3.5 days, exactly what Dr. Lyon noted in group 3. This is really an important figure brought out by these discussions.

This is the end result of our efforts as viewed by third-party payers.

Dr. Lyon's presentation should be taken as a personal challenge to each of us as it applies to our surgical techniques. This is the age of increasing demand for better results and cost-effective methods. Attention to details of surgical technique, operative routines, suture choice, and asepsis take only time and thought. We cannot depend on antibiotics to cover poor technique. It is incumbent on each of us to take Dr. Lyon's messages home and apply them in our practices.

Dr. Ralph A. Tillman, Lawrenceville, Georgia. I would like to ask Dr. Lyon one question. Do they use the Bovi for hemostasis at all at Piedmont Hospital? It certainly has been my experience that quite frequently the overuse of the Bovi is the cause of postoperative morbidity. I would like to hear a comment on that.

Dr. Edgar C. Garber, Jr., Fayetteville, North Carolina. I have not been an active obstetrician for many years but I still deal with the residents. I try to preach three things in the operating room on cesarean section and I am not very successful. Number one is expression of the placenta. As soon as the baby is delivered a resident is trying to remove the placenta. A small bolus of oxytocin will separate the placenta with very little blood loss. Second, it seems the residents cannot sew up the uterus unless it is out of the abdomen. They exteriorize the uterus, which exposes it to the room air and respiratory infections of the operating room personnel. It also places a lot of stretch on the vessels. Third, as Dr. Lyon already mentioned, the technique of closure of the uterus is important. In closing the endopelvic fascia the residents tend to use large bites and elaborate stitches with 1-0 chromic catgut. I feel this is a very timely article and I appreciate its presentation.

Dr. Lyon (Closing). Dr. Wein, thank you for your fine discussion and also for a demonstration of what a good private obstetric service can accomplish in reducing post–cesarean section morbidity. One difficulty in evaluating results is the lack of information about private obstetrics in the United States. We need enough statistics from all over the country to set standards for private service, but from what we know now your rate is excellent.

The bladder certainly does fill during the procedure and it is drained with a 16-gauge needle attached to the suction device inserted into the bladder in any retroperitoneal area. This is done as often as necessary and at closing to prevent overfilling before complete recovery from the epidural anesthetic. If the patient cannot void in the early postoperative period, a small straight catheter is used, but less than 10% of patients will require this even one time.

Postoperative hospitalization is affected by many factors in obstetric patients. Most of our patients could leave the hospital on the second postoperative morning if physical recovery alone was considered. It has been obvious that as our technique has improved postoperative recovery has become very rapid.

Thank you, Dr. Tillman, for your raising the question of Bovi hemostasis. There is no question that using the Bovi in our patients causes much more tissue reaction in the incision and subcutaneous tissue than ligation of individual vessels with fine suture. We have compared this in different patients and on different sides of the same incision. I confess though, in the interest of speed, we often use the Bovi and try to leave the smallest possible burn on the vessel.

Introduction by Dr. Leo Dunn

I am very pleased to introduce to you an old friend and distinguished colleague of mine, Dr. Henry A. Thiede, who is well known in the South for his years of Chairmanship at the University of Mississippi and in the North for his Chairmanship at the University of Rochester, where he followed Dr. Curtis Lund, one of the best known and most beloved individuals in our specialty. Dr. Thiede has carried on the traditions of teaching that Dr. Lund and his colleague John Donavan began at that institution many years ago. Dr. Thiede has served in many important positions in our specialty. He has been Director of the American Board of Obstetrics and Gynecology. He sits on the Board of Emergency Medicine. He has been a past Chairman of the Residency Review Committee and he has now taken the position of Dr. Mattingly as the Secretary of the American Gynecological and Obstetrical Society. Dr. Thiede, we apologize for all the difficulty that you had in coming here but we look forward to your comments this morning.

Urogynecology: Comments and caveats

Henry A. Thiede, M.D., and Vasant D. Saini, Ph.D.
Rochester, New York

Historically, the routine workup of the incontinent woman has focused on clinical parameters including history, pelvic examination, cotton swab test, and radiographic imaging of the bladder and urethra. Data are presented to support the concept that urinary symptoms and pelvic findings do not accurately define the causes of incontinence. Because urinary incontinence is both a dysfunctional and a structural problem, evaluation of the urodynamic response of the lower urinary tract should be part of the workup of the incontinent woman. (Am J Obstet Gynecol 1987;157:563-8.)

Key words: Stress incontinence, urodynamics, urethral closure pressure

Countrywide, most obstetrician-gynecologists employ clinical and anatomic assessments as the only means for establishing the diagnosis and deciding the treatment of women with the complaint of urinary incontinence. The problem is that female urinary incontinence is the result of abnormal function that may only coincidentally be associated with, but not necessarily caused by, abnormal pelvic support. There are a number of different reasons for stress incontinence that have no relationship to anatomy per se. We hope the examples presented here will help encourage more physicians to pursue a functional evaluation of the lower urinary tract in addition to the traditional structural evaluation.

From the Department of Obstetrics-Gynecology, University of Rochester School of Medicine and Dentistry.
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Reprint requests: Henry A. Thiede, M.D., Department of Obstetrics-Gynecology, University of Rochester, 601 Elmwood Ave., Rochester, NY 14642.

Table I. Urodynamic diagnosis of 200 women with urinary incontinence

Diagnosis	*No./%*
Genuine stress incontinence only	132/66
Detrusor instability only	28/14
Both genuine stress incontinence and detrusor instability	36/18
Other	4/2
Total	200/100

Methods and material

Two hundred women were referred to the Urogynecology Laboratory in the Obstetrics-Gynecology Department for symptoms of urinary incontinence. All patients were evaluated similarly and the data were entered on-line into an elaborate computer program described elsewhere.[1] An extensive urologic and medical history was obtained similar to that described by Ostergard.[2] Pelvic and neurologic examinations were recorded. Residual urine, urethral angles, urinalysis, urine culture, and a 24-hour voiding diary were routinely obtained. Simultaneous continuous pressure recordings of the bladder, urethra, and vagina were dis-

Table II. Percent frequency of urinary symptoms by type of incontinence

Urinary symptom	*GSI*	*DI*	*GSI and DI*
Loses with cough	84	64	92
Urgency	65	86	75
Sudden awareness	45	75*	61
Loses enroute toilet	61	89*	86*
Loses with walking	65	71	70
Loses while supine	21	29	47*
Loses with position change	38	39	58
Nocturnal enuresis	14	25	25
Riding in car increases symptoms	23	29	25
Dribbling after voiding	61	86	69

GSI, genuine stress incontinence; DI, detrusor instability.
*$p < 0.01$ compared with GSI using χ^2 test.

Table III. Degree of cystocele by urodynamic diagnosis

Degree	*GSI (No./%)**	*DI (No./%)*	*GSI and DI (No./%)*
None	6/5	5/19	6/17
1	48/39	11/42	15/43
2	61/49	8/31	9/26
3	9/7	2/8	5/14
Total	124/100	26/100	35/100

GSI, genuine stress incontinence; DI, detrusor instability.
*$p < 0.01$ when GSI was compared with DI using χ^2 test.

Table IV. Urethral axis while supine at rest by urodynamic diagnosis

Degree from horizontal	*GSI (No./%)**	*DI (No./%)*	*GSI and DI (No./%)*
<10	18/19	6/30	5/16
10-30	50/52	11/55	19/57
30-50	19/20	3/15	8/24
≥50	9/9	0/0	1/3
Total	96/100	20/100	33/100

GSI, genuine stress incontinence; DI, detrusor instability.
*$p < 0.01$ when GSI is compared with DI using χ^2 test.

played on a six-channel recorder (Disa 2100 Urosystem, Dantek Elektronik, Skovlunde, Denmark) and stored on an IBM XT computer using 8F microtip catheter transducers (Millar Instruments, Inc., Houston, Texas). The bladder was filled retrograde at 30 ml/min with sterile saline solution at 37° C. Urethral pressure profiles and resting and stress pressures were obtained at ≤100 ml in a supine position, 250 ml in a supine position, full in a supine position, and sitting erect, full with the transducer oriented to 3 o'clock. Standing pressures were obtained when indicated. Monitored and unmonitored uroflow curves were recorded; CO_2 urethroscopy with filling at 150 cc/min was performed during the same visit.

Results

The urodynamic diagnoses for the 200 incontinent women are listed in Table I. Comparison of the urinary symptoms in each of the diagnostic groups reveals that although some symptoms occur significantly more often in one group than in another, there is so much overlap that it is clear that symptoms alone cannot define a specific diagnosis for urinary incontinence (Table II). Similarly, in Tables III and IV, although the degree of cystocele and the urethral axis are significantly different ($p = <0.01$) in patients with only genuine stress incontinence when compared with patients with only detrusor instability, there is so much overlap that these observations are also of little value as tools for making a definitive clinical diagnosis.

Six patients have been selected to illustrate the importance of a urodynamic evaluation (Table V). The first two patients have a normal pelvic examination and a normal cotton swab test. S. B. (Table VI) is continent. Note, however, that in spite of her normal anatomic structure she does demonstrate a marked decrease in urethral closure pressure with Valsalva. She maintains continence because the high resting closure pressure provides a tolerance that keeps the stress closure pressure above zero and the urethral sphincter mechanism responds appropriately to filling and position change as seen in the steady level of maximum resting closure pressure. In spite of normal pelvic support, F. C. (Table VII) has genuine stress incontinence. Note the consistently low urethral closure pressure and functional area, which are maintained with bladder filling and moving to an erect position. Because she has a negative stress pressure ratio,* with the low resting urethral pressure, the urethral closure pressure drops below zero with cough or Valsalva and urine escapes. Because

*For the purpose of this presentation, a negative stress pressure ratio is a urethral pressure/bladder pressure that is <1.0. A positive stress pressure ratio is ≥1.0.

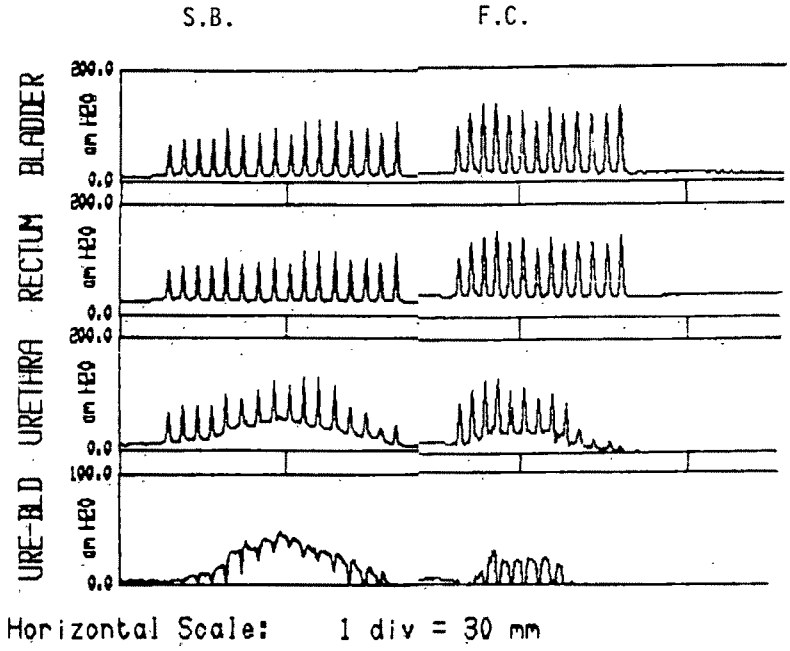


Fig. 1. The area under the curve in the URE-BLD channel of these stress urethral pressure profiles indicates the relative margin of continence. S. B. is continent; F. C. is not.

Table V. Patient summaries

Patient	*Age (yr)*	*Parity*	*Loss*	*Cotton swab test (degrees)*	*Cystocele*	*UCP*	*SPR*
S. B.	38	0	No	10	None	Normal	Negative
F. C.	43	3	Yes	10	None	Low	Negative
P. C.	32	4	No	50	None	Normal	Negative
L. T.	42	3	Yes	60	None	Low	Negative
S. R.	43	5	No	80	Moderate	Normal	Positive
A. C.	43	3	Yes	40	Moderate	Normal	Negative

UCP, resting urethral closure pressure; SPR, stress pressure ratio.

Table VI. Data for S. B.

Urethral	*Supine empty*	*Supine full*	*Sitting full*
Functional area (mm H_2O)	1059	908	711
Functional length (mm)	30	30	24
Maximum UCP (cm H_2O)	59	52	58
Valsalva UCP (cm H_2O)	15	34	28

UCP, urethral closure pressure.

Table VII. Data for F. C.

Urethral	*Supine empty*	*Supine full*	*Sitting full*
Functional area (mm H_2O)	442	340	350
Functional length (mm)	20	18	20
Maximum UCP (cm H_2O)	28	25	28
Valsalva UCP (cm H_2O)	(−)4	(−)5	(−)1

UCP, urethral closure pressure.

there is no anatomic relaxation and the urethrovesical junction is maintained in its normal abdominal position, the negative stress pressure ratio is the result of an inadequate response of the components of urethral pressure (i.e., the striated and smooth muscle and possibly also the submucosal vascularity). The inadequacy of these components also accounts for the low urethral closure pressure. The stress urethral pressure profiles for these two patients are seen in Fig. 1. Note that in the incontinent patient (F. C.) there is no functional area above zero in the urethral closure pressure *(URE-BLD)*.

Both of the two patients in the second set have an abnormal cotton swab test result but no significant cys-

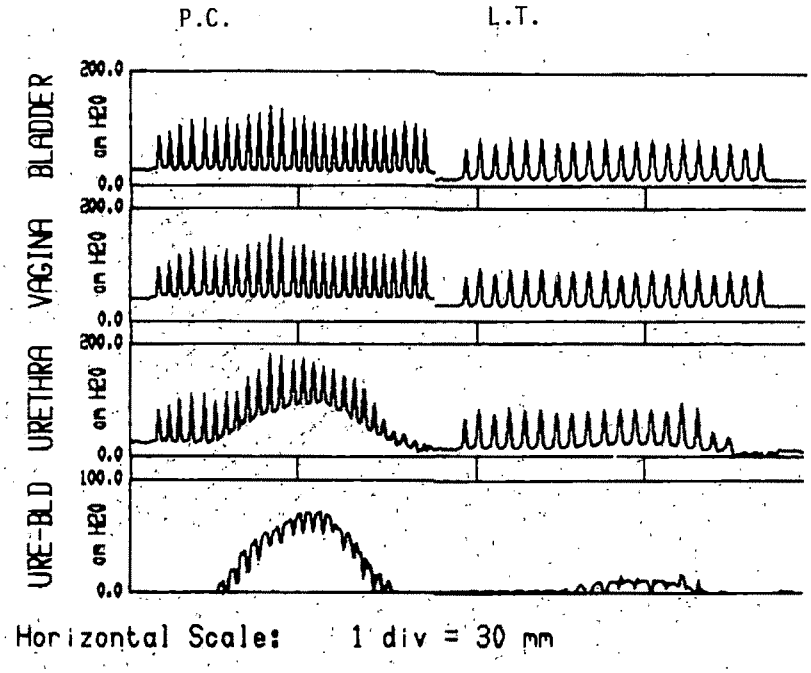


Fig. 2. These stress urethral pressure profiles illustrate the significance of a high urethral closure pressure. P. C. is continent; L. T. is not. Both have significant loss of pubourethral support.

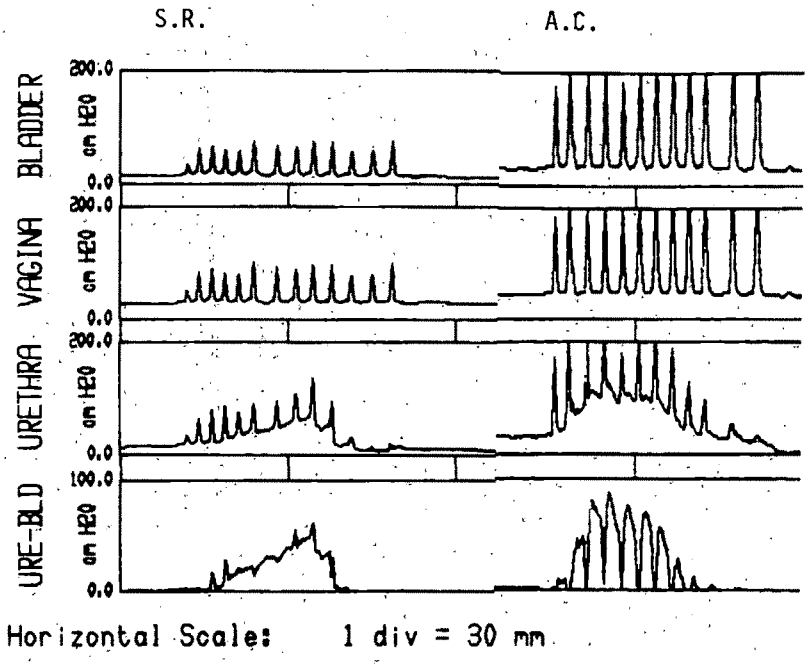


Fig. 3. The stress urethral pressure profile for S. R. is consistent with significant urethral funneling. In spite of a good urethral closure pressure, the amplitude of the stress and the loss of sphincter competence cause A. C. to be incontinent.

tocele. P. C. (Table VIII) does not lose urine in spite of loss of pubourethral support and a negative stress pressure ratio. This is because she has a high urethral closure pressure and maintains a steady functional urethral area during filling and position change. This gives her a large margin for a drop in closure pressure with stress. On the other hand, L. T. (Table IX) has a low resting urethral closure pressure and a functional urethral area that drops further during filling and position change, giving her no tolerance for the decreased pres-

Table VIII. Data for P. C.

Urethral	*Supine empty*	*Supine full*	*Sitting full*
Functional area (mm H_2O)	974	856	769
Functional length (mm)	35	28	22
Maximum UCP (cm H_2O)	53	69	50
Valsalva UCP (cm H_2O)	25	36	18

UCP, urethral closure pressure.

Table IX. Data for L. T.

Urethral	*Supine empty*	*Supine full*	*Sitting full*
Functional area (mm H_2O)	360	209	168
Functional length (mm)	25	21	10
Maximum UCP (cm H_2O)	25	22	23
Valsalva UCP (cm H_2O)	(−)2	(−)3	(−)10

UCP, urethral closure pressure.

Table X. Data for S. R.

Urethral	*Supine empty*	*Supine full*	*Sitting full*
Functional area (mm H_2O)	719	701	762
Functional length (mm)	27	23	18
Maximum UCP (cm H_2O)	39	53	51
Valsalva UCP (cm H_2O)	70	52	54

UCP, urethral closure pressure.

Table XI. Data for A. C.

Urethral	*Supine empty*	*Supine full*	*Sitting full*
Functional area (mm H_2O)	744	545	603
Functional length (mm)	28	21	19
Maximum UCP (cm H_2O)	72	60	60
Valsalva UCP (cm H_2O)	(−)9	(−)10	3

UCP, urethral closure pressure.

sure in the urethra with stress; hence, significant stress incontinence occurs. The stress urethral pressure profiles for these two patients are seen in Fig. 2. In L. T. there is hardly a perceptible tracing in the subtracted channel *(URE-BLD)*.

The last two patients have both an abnormal cotton swab test result and a moderate (second degree) cystocele. Both patients have normal closure pressures that are maintained with filling and position change. However, S. R. (Table X) maintains a functional urethral area and also has a positive stress pressure ratio in spite of her anatomic defect. Additionally, her urethral pressure profile is consistent with urethral funneling, which was subsequently demonstrated on a voiding cystourethrogram. She maintains urinary continence in face of the anatomic defect because the urethra is a dynamic structure and the elements compromising her urethral pressure reacted positively to the sudden increase in abdominal pressure. On the other hand, A. C. (Table XI) has some attrition of the resting functional urethral area with filling because of shortening of the functional length of the urethra. Although it is a symmetric urethral pressure profile, note the marked negative stress pressure ratio that is the result of the very large increases in intra-abdominal pressure and the inability of the urethra to compensate functionally for this with leakage as a result (Fig. 3).

Comment

Cardozo and Stanton[5] found that urinary symptoms and the degree of pelvic relaxation do not discriminate

Table XII. Elements of urinary continence detectable only by urodynamic evaluation

Amplitude of UCP
Widely fluctuating UCP
Response of UCP to increased abdominal pressure
Detrusor contraction occurring in immediate proximity to sudden increase in abdominal pressure
Abnormal vesical compliance
Pressure-flow relationship and voiding pattern
Contribution to UCP of large cystocele, rectocele, or procidentia
Chronic urethritis

UCP, urethral closure pressure.

patients with genuine stress incontinence from patients with detrusor instability. In their prospective study of the cotton swab test, Fantl et al.[4] found that assessment of the urethral axis was not predictive of urethral function. The six cases reported in this study also illustrate that it was not the anatomic support but the ability of the urethra to respond to sudden increases in abdominal pressure and the level of tolerance in urethral closure pressure that determined continence. Table XII lists factors that affect continence but are not apparent except by urodynamic evaluation.

Urodynamic workup often uncovers genuine stress incontinence resulting from caffeine abuse, hypoestrogenic atrophy, urethrocystitis, prescription drugs such as phenothiazines, antihistamines, and calcium channel blockers, and neurologic disorders, to name a few. Any of these may affect the ability of the urethra to respond

appropriately to a sudden increase in abdominal pressure or may contribute to a low resting urethral closure pressure, which lowers the tolerance for any significant negative stress pressure ratio. If present, anatomic relaxation can be merely coincidental; surgery certainly is not the primary treatment under those circumstances.

Urodynamic studies that do not include simultaneous monitoring of urethral, bladder, and abdominal pressures and the capability of reading urethral closure pressure and detrusor pressure (as opposed to bladder pressure) are likely to be misleading. There is no room for shortcuts; no single urodynamic study or parameter has been found that can give a reliable diagnosis. Compulsive attention to many details is essential for the urodynamic results to be valid, and the studies probably should not be undertaken by the occasional participant.

Having said that, it is also important to state that urodynamic techniques and interpretations are still evolving; the reproducibility, sensitivity, and specificity of some aspects have yet to be evaluated. Urodynamics is a rapidly growing research area that lends itself to the application of the sophisticated new technology that is becoming available. However, even in its present form it is a valuable if not essential tool for the clinician in the workup of urinary incontinence.

We gratefully acknowledge the contributions and assistance provided by Alean Fullerton, B.S.N., Kathy Schwarz, R.N., M.S., and Florence Thiede, B.S.N.

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Necrotizing fasciitis of the vulva

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Sixteen cases of necrotizing fasciitis arising from or involving the vulva are presented. Fifteen of the 16 women had diabetes mellitus. Five of them died. All but one of these cases were managed by very extensive excision of all involved tissues. These cases demonstrate the extreme seriousness of any infection involving the vulva in a diabetic patient. Such patients should have the infected area explored while they are under anesthesia as soon as possible. If any necrosis is found, radical excision to include removal of all necrotic, indurated, and pale tissues is mandatory. (AM J OBSTET GYNECOL 1987;157:568-71.)

Key words: Necrotizing fasciitis, synergistic bacterial infection, diabetes mellitus, radical excision

Necrotizing fasciitis is a rapidly progressive, highly toxic, often fatal, and commonly synergistic bacterial infection. Usually, it arises after minor trauma or an insignificant-appearing infection. Necrotizing fasciitis, as a complication of infections of the vulva and perineal region, is fortunately quite rare.

The initial signs of necrotizing fasciitis are erythema, edema, and localized pain. When areas of dark discoloration, bullae, or obvious skin necrosis develop, there will always be extensive underlying necrosis and the infection may spread quickly along fascial planes with resultant necrosis of large areas of the body. Diabetic patients with any abscess or infection of the vulva or perineal area need immediate hospitalization and aggressive surgical management. When there is evidence of necrosis, incision and drainage and antibiotic therapy are insufficient treatment. Although reports of necrotizing fasciitis in other areas of the body do not indicate that diabetes mellitus is an integral part of the pathogenesis of the disease, diabetes mellitus is nearly always present in the patient with necrotizing infections involving the vulva.

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Table I. Extent of disease, initial surgical management, and outcome

Patient No.	*Age (yr)*	*Previous diabetic management*	*Initial blood glucose (mg/dl)*	*Extent of necrosis*	*Initial surgical procedure*	*Outcome*
1	40	None	697	Vulva, femoral triangle, ischiorectal fossa	Incision and drainage	Recovered
2	54	Diet	435	Left labium majus and groin	Wide excision	Recovered
3	64	Insulin	730	Right labia, groin to iliac crest, and medial thigh	Wide excision	Recovered
4	37	Diet	361	Left labia, femoral triangle	Wide excision	Recovered
5	69	Diet	775	Vulva, right buttock, ischiorectal fossa, entire right leg	Incision and drainage	Died
6	85	Diet	473	Right labium majus, groin, and medial thigh	Wide excision	Died
7	62	Oral hypoglycemics	568	Left labia and groin	Incision and drainage	Recovered
8	61	Nondiabetic	55	Right labia, buttock, and medial thigh	Wide excision	Recovered
9	40	Diet	116	Left groin and medial thigh	Extensive debridement	Recovered
10	79	Oral hypoglycemics	150	Vulva, both sides of groin, upper thighs	Extensive debridement	Died
11	83	None	94	Right labia and medial thigh	Wide excision	Died
12	55	Oral hypoglycemics	272	Left labium majus and medial thigh	Wide excision	Recovered
13	55	None	332	Right labium majus, groin, ischiorectal fossa	Wide excision	Recovered
14	42	Oral hypoglycemics	331	Left labial and medial thigh	Wide excision	Recovered
15	62	None	560	Left labia, buttock, and medial thigh	Wide excision	Died
16	65	Diet	439	Vulva, buttocks, both legs, trunk to axillae	Debridement	Recovered

Historical review

In 1926 Brewer and Meleney[1-3] reported two cases of progressive gangrenous infection of the skin and subcutaneous tissues that occurred in and around abdominal wounds after operation for acute perforative appendicitis. The wound infections were stated to resemble carbuncles; the skin edges had early necrosis, and the skin was undermined with rather extensive necrosis. The first patient was treated by creation of a deep circumferential incision that enclosed the diseased area, and the encircling wound was packed. The enclosed area sloughed, but the infection did not extend beyond the barrier. The second patient was cured by wide excision of the area of infection and necrosis. Animal cultures showed that the infection was synergistic and that one organism alone was not able to produce the progressive necrosis seen in the two patients. Their study also provided the basis for management.

In 1952 Wilson[4] reported 22 cases of necrotizing fasciitis at Parkland Hospital in Dallas, Texas, and found that, when probing demonstrated undermining, immediate surgical treatment was indicated. He stated: "... to postpone surgery and use massive doses of antibiotics is ineffective." In 1972, Roberts and Hester[5] reported six cases of necrotizing fasciitis involving the

Table II. Bacteria cultured

Organism	*No.*
Bacteroides sp.	3
Coagulase-negative streptococci	3
Enterococcus	3
Escherichia coli	3
β-Hemolytic streptococci, group A	2
Peptococcus	2
Proteus mirabilis	2
Diphtheroids	1
Fusobacterium symbiocum	1
Klebsiella	1
Pseudomonas aeruginosa	1
Staphylococcus aureus	1
Streptococcus viridans	1
No growth	1

vulva, all in diabetic women. In that series of cases three of the four patients whose initial surgical procedure was incision and drainage or debridement died, while the other was left disabled. Two had wide excision as the initial surgical procedure, and both were cured. The authors concluded that "Patients with diabetes mellitus, regardless of severity, and abscesses of the vulva or Bartholin's gland duct must be considered to have necrotizing fasciitis until proved otherwise. Such patients should have the vulva explored under anesthesia, and, if tissue necrosis is found, radical excision to include removal of all necrotic and indurated tissues be performed . . .."

Patient review

Sixteen additional cases of necrotizing fasciitis involving the vulva are presented (Table I). Only one patient was not diabetic. Fourteen presented with either a history of diabetes mellitus or had an elevated blood glucose level at the time of admission. Patient No. 11 had an initial blood glucose level of 94 mg/dl but soon required insulin.

Most of the patients were not aware of the origin of the infection, although some began as Bartholin's gland duct abscesses. There were five deaths and these deserve review. In Case No. 5, the patient was managed initially in the emergency room where she had incision and drainage of a Bartholin's gland duct abscess. Five days later she was admitted in ketoacidotic coma with extensive necrosis of the skin and subcutaneous tissues of the lower abdominal wall, both sides of the groin, the entire vulva, the right buttock, and the entire right leg. She initially had rapid improvement in the blood glucose level, became alert, and seemed to respond to radical excision of the skin and subcutaneous tissues of the lower abdomen, radical vulvectomy, and disarticulation of the right hip. She then died of a sudden cardiac arrhythmia. In Case No. 6, the patient apparently had a myocardial infarction and collapsed in a bed of fire ants. When found she was unconscious and had extensive necrotizing fasciitis of the right labium, groin, and medial thigh. She died 2 days after admission and subsequent radical excision of all involved tissues. Autopsy results revealed myocardial infarction and necrotizing enterocolitis. In Case No. 10, the patient died of sepsis in spite of early extensive debridement and multiple-agent antibiotic therapy. In Case No. 11, the patient developed renal failure and died of azotemia and sepsis. Stone and Martin[6] reported nearly a 100% death rate in their diabetic patients with severe renal disease. In Case No. 15, the patient died suddenly, apparently of a pulmonary embolus, after nearly complete healing of the wound.

Case No. 16 is worthy of review as fascial and subcutaneous necrosis were found to involve the entire vulva, both sides of the groin, both buttocks and both legs, the trunk, and both axillae. Extensive debridement of areas of necrosis of the vulva and groin was performed. The patient was treated with multiple antibiotics and recovered even though the involved areas could not be surgically removed.

Bacteriology

Seven cases had more than one organism cultured from the areas of involvement (Table II). Many of these patients had been treated with antibiotics prior to being diagnosed as having necrotizing fasciitis, which probably interfered with efforts to obtain accurate data. The progressive necrosis seen in these cases is consistent with the belief that these are synergistic infections.

Comment

Necrotizing fasciitis involving the vulva is thought to be rare but 16 cases from a community hospital are reported, suggesting that the entity occurs more frequently than previously expected. Of the 22 patients presented in my two articles, all were adults and all but one had diabetes mellitus. Necrosis is a late sign and by the time skin necrosis is present the underlying liquefaction necrosis will have involved a very large area.

Initially, necrotizing fasciitis may appear as a simple abscess, but in the compromised patient this appearance is deceptive. Obvious necrosis is a late sign; by the time skin necrosis is present the underlying necrosis will have extended and involve far more tissue than is evident from examination. Systemic signs of toxicity are a late feature. No one group of bacteria is responsible for this disease, and the disease can be caused by almost any aerobic or anaerobic pathogen, either alone or synergistically.

Prompt recognition, resuscitation, immediate operation including complete and wide excision, antibiotic therapy, and multisystem support can be lifesaving. Excision should include removal of all diseased tissues until bleeding is encountered. Any tissues that do not have a pink color suggesting good capillary perfusion should be removed. The wounds can be managed most efficiently by leaving the defect open. Wounds can later be skin grafted and covered by a rotational flap graft; if kept clean the wounds will gradually close with a very narrow scar that often will give the appearance of a primary closure.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Henry A. Thiede, Rochester, New York. The mortality rate in Dr. Roberts' series of 16 cases is 31% which compares favorably with the much higher mortality rates of other series. Early diagnosis makes the difference, but this is sometimes difficult, even if necrotizing fasciitis is under consideration. The overlying skin varies in appearance. It may be red or pale or cyanotic with bullae and gangrene. The margins may be distinct or vague. In advanced disease there is extensive undermining of the skin and a four-smelling sanguineous discharge sometimes described as "dishwater" in appearance. The superficial and deep fascia can be separated easily and appear stringy and gray-green in color. In the early stages of the disease, when cure is most likely, these findings may not be so dramatic. The use of frozen-section biopsy of the fascia to make a definitive diagnosis in the early evolution of the disease is championed by some and is worthy of consideration.

Survival in patients with necrotizing fasciitis of the vulva depends on early, extensive and complete debridement of the necrotic and infected tissues. An addendum to Dr. Roberts' fine description of the surgical management is the need to examine the operative area thoroughly, about 24 hours after the initial debridement, for any areas of persistent necrosis that may have escaped notice or developed in the interim. It is often necessary to anesthetize the patient again to effect this examination. Given that the patient may very well be prostrate from sepsis, this can be a difficult but important decision. Continued close observation of the wound is necessary. There is no conclusive evidence that topical antibiotics or wet dressings, dry dressings, or honey dressings are significantly superior. Amniotic membranes have been used as a temporary wound dressing in large defects. The effectiveness of hyperbaric treatment and hyperalimentation in these patients is still under study.

This paper deals with necrotizing fasciitis of the vulva and stresses that it is a diagnosis to be excluded in any diabetic patient with a vulvar infection. This is a good concept. However, obstetrician-gynecologists may be seeing more of this entity as a postoperative complication with an aging population that has arteriosclerosis and other chronic debilitating diseases, such as cirrhosis, renal failure, cancer, and malnutrition, that compromise host defenses. We are also seeing a growing number of younger women with drug dependency and immunosuppression caused by human T-lymphotropic virus type III, all of which are thought to predispose patients to an increased risk of necrotizing fasciitis. Cases have been reported after laparoscopy, abdominal hysterectomy, episiotomy, and vulvectomy. Moreover, the newborn infant is no exception as necrotizing fasciitis has been described as a complication of omphalitis, urachal anomalies, and necrotizing enterocolitis.

The compromised host defense also impacts on the selection and use of antibiotics. Like Dr. Roberts, most authors report mixed infections. For many years it was taught that necrotizing fasciitis was the result of a synergistic infection between hemolytic staphylococci and anaerobic streptococci. While these organisms are frequently present, gram-negative enteric bacteria and *Bacteroides* are also frequent isolates. *Clostridium* is occasionally present.

Cultures are important in selecting the correct antibiotic therapy. However, until the isolates and their sensitivities are reported, usually an interval no less than 48 hours, it seems appropriate to start the patient initially on a regimen of broad spectrum therapy such as a penicillin or cephalosporin, gentamicin and clindomycin, or metronidazole. A gram-stained smear of the exudate may occasionally give an early indication before culture of the presence of clostridium and should be routine.

As important as antibiotics and intensive care are, only an occasional patient survives if extensive debridement of the necrotic and infected tissue is not effected promptly. This and early diagnosis, which is usually the result of a heightened awareness of the disease process, will save lives. We thank Dr. Roberts for his contribution to this literature.

Outcomes in patients with unusually high maternal serum α-fetoprotein levels

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In a study group of 166 patients with unusually high maternal serum α-fetoprotein values ≥5 multiples of the median, 110 (66%) patients had a condition affecting obstetric care compared with 14% in the 2.5 to 2.9 range, 26% in the 3.0 to 3.9 range, and 30% in the 4 to 4.9 range of multiples of the median. Fetal anomalies composed a significantly greater proportion ($p < 0.0001$) of positive findings in the study group than the group with maternal serum α-fetoprotein values of ≥2.5 to 4.9 multiples of the median. Fetal death either before 20 weeks or of one twin occurred significantly more often in the study group ($p < 0.0001$). Neural tube defects (21%) and fetal death before 20 weeks (19%) were the most common findings in the study group. There was not a statistically significant difference ($p < 0.53$) in pregnancy complications or in late complications between the two groups although oligohydramnios and abdominal pregnancies occurred more often in the study group ($p < 0.029$ and < 0.011). Diagnosticians evaluating patients with unusually elevated maternal serum α-fetoprotein values must be aware of the usual differential diagnosis as well as rarer causes. One must recognize that finding an unaffected fetus does not preclude the subsequent development of a pregnancy complication. (Am J Obstet Gynecol 1987;157:572-6.)

Key words: α-Fetoprotein, ultrasound

Screening programs using maternal serum α-fetoprotein levels have been described in several reports.[1-3] As experience increases, subgroups of patients will be identified, for example, patients with low maternal serum α-fetoprotein values,[4] in whom there is a greater risk of findings that could impact on obstetric care. The purpose of this article is to present outcomes in a group of patients with unusually high maternal serum α-fetoprotein values.

Method

The procedures followed in this screening program have been described.[1] An elevated maternal serum α-fetoprotein value was defined as ≥2.5 multiples of the median and was adjusted for maternal weight and diabetes mellitus. Patients in whom a sample of maternal serum α-fetoprotein equaled or exceeded 5 multiples of the median were identified and monitored for outcome. This subgroup was selected by determining the lowest multiple of the median above which 50% or more of the population had a positive finding. A positive finding was defined as any diagnosis that could adversely affect pregnancy outcome. For analysis and discussion, the results were grouped into the following categories: (1) fetal anomalies (neural tube defects, gastroschisis, omphalocele, triploidy); (2) fetal death (fetal death before 20 weeks, fetal death of one twin); (3) pregnancy complications (twins, oligohydramnios, abdominal pregnancies, placental hemangioma); and (4) late complications (subsequent obstetric complications and fetal death after 20 weeks). Those conditions more related to the maternal-fetal unit such as fetal distress, toxemia, intrauterine growth impairment, premature labor, abruptio placentae, or premature amniorrhexis were grouped under subsequent obstetric complications. Twins were considered as a positive find-

Table I. Number of patients in each multiples of the median range and the frequency of findings, including twins

Range	Total No. of patients	Positive findings	
		n	%
2.5-2.9	884	124	14
3.0-3.9	410	104	25
4.0-4.9	97	29	30
5.0-5.9	52	28	54
6.0-6.9	24	12	50
7.0-7.9	25	15	60
8.0-8.9	15	11	73
9.0-9.9	8	7	88
≥10	42	37	88
Total	1557	367	24

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Table II. Frequency of positive findings in the 166 patients with maternal serum α-fetoprotein values ≥5 multiples of the median

Finding	*Multiples of the median range*						*Subtotal*	*% of total*
	5-5.9	*6-6.9*	*7-7.9*	*8-8.9*	*9-9.9*	*>10*		
Fetal anomaly							49	29
Neural tube defect	10	2	5	5	3	10	35	21
Gastroschisis	–	1	2	1	1	2	7	4
Omphalocele	–	–	–	–	1	4	5	3
Triploidy	–	–	1	–	1	–	2	1
Fetal death							34	20
Before 20 wk	8	4	4	2	–	14	32	19
Of one twin	–	–	–	–	–	2	2	1
Pregnancy complication							15	10
Twins	5	1	–	–	–	–	6	4
Oligohydramnios	3	–	1	1	1	–	6	4
Abdominal pregnancy	–	–	–	–	–	2	2	1
Placental hemangioma	–	–	–	–	–	1	1	1
Late complication							12	7
Subsequent obstetric complication	2	3	1	2	–	1	9	5
Fetal death after 20 wk	–	1	1	–	–	1	3	2
Total	28	12	15	11	7	37	110	66

Table III. Occurrence of positive findings in the study group (≥5.0 multiples of the median) compared with the total group with two elevated maternal serum α-fetoprotein values (≥2.5 multiples of the median)

	≥2.5 to 4.5 multiples of the median		*Study group (≥5 multiples of the median)*		*Elevated group (≥2.5 multiples of the median)*		*p Value**
	n	%	*n*	%	*n*	%	
No.	1391		166		1557		
Fetal anomaly	23	2	49	30	72	5	<0.0001
Neural tube defect	23	2	35	21	58	4	<0.0001
Gastroschisis	0	0	7	4	7	0.4	<0.001
Omphalocele	0	0	5	3	5	0.3	<0.001
Triploidy	0	0	2	1	2	0.1	<0.011
Fetal death	16	1	34	20	50	32	<0.0001
Before 20 wk	15	1	32	19	47	3	<0.001
Of one twin	1	0.1	2	1	3	0.2	<0.032
Pregnancy complication	129	9	15	10	144	9.7	<0.53
Twins	112	8	6	4	118	8	<0.22
Oligohydramnios	17	1	6	4	23	1.5	<0.029
Abdominal pregnancy	0	0	2	1	2	0.1	<0.011
Placental hemangioma	0	0	1	1	1	0.1	<0.099
Late complication	89	6	12	7	101	6.9	<0.38
Subsequent obstetric complication	78	6	9	5	87	6	<0.55
Fetal death after 20 wk	11	0.8	3	2	14	0.9	<0.18
Total	257	18	110	66	367	24	<0.001

*Comparison made between those findings in the ≥2.5 to 4.9 group and the ≥5 group.

ing and any obstetric complication affecting twins was omitted from the data unless the condition was a neural tube defect, in which case it was counted. Patients were followed up until completion of pregnancy and the patient and physician were contacted for outcome data. Statistical analysis was by Fisher's exact χ^2 test (one-tailed).

Results

Between July 1978 and September 30, 1986, 46,051 patients were screened and there were 1557 (3.4%) who had a first elevation and for whom follow-up data have been completed. Of these, 166 patients had an unusually high maternal serum α-fetoprotein value. The percentages of positive findings for the lower maternal

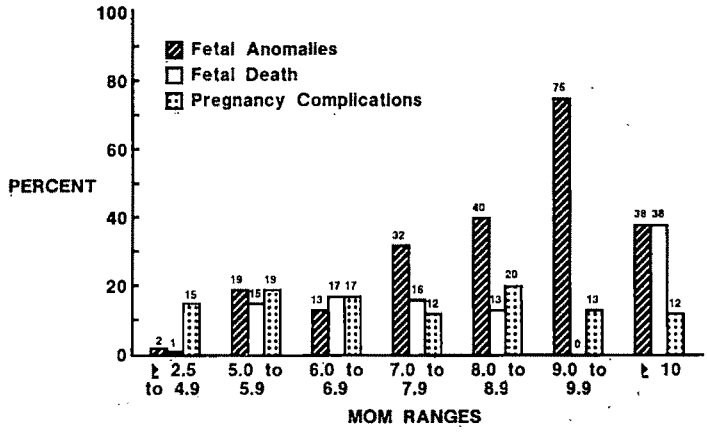


Fig. 1. Distribution of findings. *MOM:* Multiples of the media

serum α-fetoprotein ranges were 14 for 2.5 to 2.9 multiples of the median, 26 for 3.0 to 3.9 multiples of the median, and 30 for 4.0 to 4.9 multiples of the median (Tables I and II). The percentages for the higher maternal serum α-fetoprotein values ranged from 50 to 88. Fetal anomalies composed a significantly greater proportion ($p < 0.0001$) of positive findings in the study group than in the group with maternal serum α-fetoprotein values of ≥2.5 to 4.9 multiples of the median (Table III). Fetal death either before 20 weeks or of one twin occurred significantly more often in the study group ($p < 0.0001$). Neural tube defects and fetal death before 20 weeks were the most common findings in the study group. There was not a statistically significant difference ($p < 0.53$) in pregnancy complications or late complications between the two groups although oligohydramnios and abdominal pregnancies occurred more often in the study group ($p < 0.029$ and < 0.011).

In a subset of the study group defined by an elevation ≥9 multiples of the median, fetal death before 20 weeks and neural tube defects remained the most common findings. The rarer conditions of omphalocele, death of one twin, abdominal pregnancy, and placental hemangioma were all in this subset.

Comment

The cutoff for defining elevated levels of maternal serum α-fetoprotein is usually arbitrarily set at 2.0 to 3.0 multiples of the median for gestational age. Elevated maternal serum α-fetoprotein levels have been associated with numerous adverse outcomes of pregnancy including fetal neural tube defects, spontaneous abortion, stillbirth, prematurity, and intrauterine growth retardation. There is considerable overlap between the distribution of maternal serum α-fetoprotein levels seen in normal pregnancy and those associated with neural tube defects and other anomalies. Many patients defined as having maternal serum α-fetoprotein elevations will have a normal outcome of pregnancy while others will have a benign explanation for the apparent elevation, such as a more advanced gestational age than anticipated or twins. In dealing with unusually high elevations, a benign explanation is rare and one might anticipate an increased frequency of adverse outcomes. Our data (Fig. 1) confirm this by demonstrating a 66% incidence of abnormal findings or adverse outcomes in patients with maternal serum α-fetoprotein elevations ≥5.0 multiples of the median and an 88% incidence in patients with maternal serum α-fetoprotein elevations ≥9.0 multiples of the median. The particularly high frequency in the 9 to 9.9 multiples of the median range is probably the effect of a small total number in that range. Of the 66% with abnormal findings, the majority were fetal anomalies (29%) and fetal death (20%). The significant differences persist for individual findings within fetal anomalies and fetal death. Although pregnancy complications appeared to occur equally in the lower ranges of maternal serum α-fetoprotein elevation and the unusually high values, the individual findings of oligohydramnios and abdominal pregnancies were more frequent in the unusually high groups. Because of the small numbers found in some individual categories, one must use caution in interpreting the data.

Clues to the causes of maternal serum α-fetoprotein values were found by studying the placenta in three of the cases and amniotic fluid volume in six. While triploidy is definitively diagnosed by amniocentesis, a thick placenta may be found at ultrasonography.[10] The other placental abnormality, a hemangioma, was also associated with increased placental mass. Placental abnormalities in patients with elevated maternal serum α-fetoprotein values have been described.[11-13] As placental size increases, the surface area across which α-fetoprotein can pass from fetal to maternal circulation also increases, theoretically resulting in higher ma-

ternal serum α-fetoprotein values. Alternatively, a breakdown in the fetal-maternal/placental barrier may be an explanation.[11] Oligohydramnios has been associated with a poor pregnancy outcome[5, 14] and will be the subject of a later report.

The study group with unusually high maternal serum α-fetoprotein was predestined to have a significant number of conditions affecting the fetus, mother, or both because the group was selected as those in whom more than 50% had significant findings. Identified in this group was a subset, those 44 patients with maternal serum α-fetoprotein values ≥9 multiples of the median, who had an 88% chance of a significant positive finding. This subset requires diligent evaluation by expert ultrasonographers and by amniocentesis.

The two elevations of 13.2 and 19.6 multiples of the median that were associated with abdominal pregnancies deserve special note. There is one previous case report of an elevated maternal serum α-fetoprotein level associated with abdominal pregnancy,[15] making these two the second and third reported cases. One group of investigators found elevated maternal serum α-fetoprotein levels in ectopic pregnancies[16] but noted only two of five values above the ninety-fifth percentile. Considering that the majority of the conditions in this series had significant findings by ultrasound alone, one should be highly suspicious of abdominal pregnancy in a patient with an unusual maternal serum α-fetoprotein elevation and normal ultrasound findings.

This article has presented a summary of pregnancy outcomes in a group of patients with unusually high maternal serum α-fetoprotein levels. Investigation of patients in this group requires thorough knowledge of possible causes, ultrasound, and other diagnostic procedures. One must recognize that finding an unaffected fetus does not preclude the subsequent development of a pregnancy complication.

We wish to acknowledge the assistance of Ms. Chris Morris in maintaining data on the serum α-fetoprotein screening program and diligently researching information for this report.

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Discussion

DR. GUY M. MEARES, JR., Columbia, South Carolina (Official Guest). I would like to commend Dr. Nelson and his associates for the information they have presented in this article.

The large and extensive screening experience is readily apparent by the sheer volume of patients who have been screened during this ongoing study.

In their group of 166 patients with >5 multiples of the median, the occurrence of abnormal outcomes in 110 patients depicts high-risk factors for this group. While the incidence of abnormalities in this group is high, the proportion of patients in this group is small relative to the total group screened.

A major problem with maternal serum α-fetoprotein screening is the elevated α-fetoprotein value that is confirmed when repeated and no abnormality can be detected after ultrasound and amniocentesis have been performed. The parental anxiety that is created in this situation may be extremely intense and may mar the couple for the remainder of the pregnancy. While the couple may be reassured from the standpoint of an anomaly, can this elevated value be serving as a warning for the advent of complications later in pregnancy?

Even more important in dealing with the anxiety as-

sociated with α-fetoprotein testing, it is incumbent on us to have timely and qualified ultrasonography and amniocentesis available when elevated α-fetoprotein levels are confirmed. I have been impressed with the number of patients who refuse to proceed with any evaluation after an elevated maternal serum α-fetoprotein level. These same individuals were completely informed of the nature of the testing before its being performed and understood what abnormalities could be present.

I agree that caution should be exercised in predicting risk percentages in groups with small numbers, as with the >9 multiples of the median group.

There are three questions I would like to pose to Dr. Nelson: (1) Why do anomalies compose a greater percentage of the study group when compared with the control group? (2) What is the mechanism of elevated α-fetoprotein levels in fetal death in utero and why does α-fetoprotein rise before fetal death? (3) What is the mechanism of elevated α-fetoprotein levels with oligohydramnios?

Discusser No. 2. I just wanted to correct a misconception about the position of the American College of Obstetricians and Gynecologists in this regard. We opposed the release of the α-fetoprotein testing kits but in spite of our good advice the Food and Drug Administration chose to release them. So the position of the College was that the fellows should be cautioned as to their liability regarding these released testing kits. There would be in the range of a couple of thousand neural tube defects and if one of these babies was born and the patient had not been advised that a test was available, the liability situation would be considerable. It was a caution based on the liability rather than the science.

Dr. Henry A. Thiede, Rochester, New York. Dr. Nelson, I recognize that you have addressed the high values, but I wonder whether you have run across any patients with extraordinarily low values in that large series who subsequently developed choriocarcinoma. Malonski and Ishaguria have indicated that there may be some cause for concern in this group of patients that, even though a fetus is present, they may subsequently be at risk for choriocarcinoma.

Dr. Nelson (Closing). Dr. Thiede, we have not had patients with high values who subsequently developed choriocarcinoma. We are experienced with the ultrasound aspect of low α-fetoprotein values (in press in the *Journal of Ultrascund in Medicine*) but we have not had that experience. To respond to Dr. Meares' questions, a high rate of abnormalities in our study group was predestined by the method by which we selected the study group. We selected a group above which 50% would have some anomalous condition. The issue of maternal serum α-fetoprotein associated with fetal deaths is interesting. Fetal death can be found both in the low group and in the elevated group. I speculate that when the baby dies, whatever the mechanism is that produces the α-fetoprotein ceases and the α-fetoprotein then decreases. As the baby becomes macerated and the skin barrier breaks down, the ability of the α-fetoprotein within the infant to diffuse into the amniotic fluid and then into the maternal circulation increases, resulting in higher levels. This question, however, was more to the point of why does it rise before fetal death? The rise may be related to an event that we are unable to detect and that produces death later on. It is not the death per se that causes the rise but the event that is going to cause the death. We cannot answer that question despite Dr. Pitkin's presentation yesterday. In oligohydramnios, why is α-fetoprotein elevated? I think it is simply a principle of diffusion. The relative α-fetoprotein levels around 16 weeks are about 2,720,000 ng in the fetus and 47 ng in the mother. Therefore I think that a higher concentration per milliliter of amniotic fluid in the infant results in a higher value in the maternal serum.

Mechanical intestinal obstruction in patients with gynecologic disease: A review of 368 patients

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To review the management of intestinal obstruction associated with gynecologic disease, the authors studied the records of 368 patients with acute intestinal obstruction. Most patients (83%) had gynecologic malignancies. Obstruction of the small intestines was more common than obstruction of the large intestines (77% versus 23%). Major causes of mechanical small bowel obstruction included extrinsic neoplasms (62%, mostly ovarian carcinomas), radiation therapy–associated strictures and adhesions (17%), postoperative adhesions (14%), and inflammatory strictures and adhesions (3%). Obstruction of the colon was caused mainly by extrinsic neoplasms (45%), strictures and adhesions associated with radiation therapy (26%), fecal impaction (9%), and intrinsic neoplasms (8%). Gastrointestinal intubation successfully relieved 81% of small bowel obstructions caused by postoperative adhesions. Tube suction alone was rarely successful when the obstruction was caused by malignant neoplasms. The prognosis was dependent on the cause of the underlying disease. The cases studied in this report were compared with a large number of cases of bowel obstruction in general surgery. It is concluded that bowel obstruction associated with gynecologic disease has unique features deserving wider recognition. (AM J OBSTET GYNECOL 1987;157:577-83.)

Key words: Mechanical intestinal obstruction, intestinal obstruction in gynecology, bowel obstruction associated with gynecologic disease, obstruction of intestines in gynecology

Although there are several reports of intestinal obstruction associated with ovarian carcinoma,[1-3] comprehensive studies of bowel involvement with diseases of the genital tract are lacking. Therefore, gynecologists must refer to the general surgical literature for guidance if they encounter blockage of the small or large intestines from causes other than ovarian carcinoma. Yet, involvement of the intestinal tract by pelvic disease or gynecologic operations is frequent enough to warrant a separate review. This report is based on experience with intestinal obstruction during a 25-year period on the gynecology service of a large teaching hospital.

Patients and methods

The records of 368 patients with acute mechanical intestinal obstructions were reviewed. The patients were hospitalized between 1960 and 1984 at the Medical College of Virginia. The age of the patients ranged from 26 to 93 years; the mean age was 52.6 years. The diagnosis of obstruction was based on history, physical examinations, and supine and upright roentgenographic films of the abdomen showing dilated loops of the bowel or air-fluid interfaces or both. In 27% of all cases, roentgenographic contrast studies of the small or large intestines, or both, were performed. The clinical diagnosis of intestinal obstruction was confirmed by exploratory laparotomy (with or without intestinal operation) or diagnostic roentgenographic studies, or both, or by autopsy.

Patients with terminal illness receiving supportive care and those with postoperative paralytic ileus were excluded from this study. Cases of incomplete obstruction of the small bowel or colon were also omitted unless there was radiographic evidence of proximal distention of the bowel. Obstruction of the small intestines and colon at the same time were classified as small bowel obstruction if dilated loops of the small bowel were present and as large bowel obstruction if portions of the colon were dilated.

Resolution of the obstruction was defined as remission of pain, vomiting, and abdominal distention, as well as passage of flatus or stool, for at least 30 days. These patients were able to tolerate oral feedings and were discharged from the hospital without surgical intervention.

Operative death is defined as death from any cause within 30 days of the operation. For comparison with a large surgical series, causes of obstruction as a result of gynecologic disease were classified in a manner similar to that described by Wangensteen.[4]

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Table I. Causes of mechanical small bowel obstruction in 283 gynecologic patients at the Medical College of Virginia: comparison with a surgical series of 706 cases[5]

Causes of obstruction	*Gynecology*		*General surgery (% of total)*
	No.	*% of total*	
Neoplasm, extrinsic	175	61.8*	9.8
Postoperative adhesions	41	14.5	40.6
Inflammatory strictures	34	12.0†	4.1
Inflammatory adhesions	23	8.1‡	6.8
External hernia	2	0.7	16.7
Neoplasm, intrinsic	0	0	5.0
Miscellaneous	8	2.9§	17.0

*Ovarian neoplasm 51.6% (malignant 49.8%, benign 1.8%), endometrial carcinoma 4.2%, cervical carcinoma 3.5%, fallopian tube carcinoma 1.8%, and uterine sarcoma 0.7%.

†Radiation therapy 10.6%, pelvic inflammatory disease and pelvic abscess 0.7%, and peritonitis 0.7%.

‡Radiation therapy 6.3%, pelvic inflammatory disease and pelvic abscess 1.1%, and peritonitis 0.7%.

§Endometriosis 1.1%, internal hernia 0.7%, intussusception 0.7%, and volvulus 0.4%.

Table II. Causes of large bowel obstruction in 85 gynecologic patients at the Medical College of Virginia: comparison with a surgical series of 393 cases[5]

Causes of obstruction	*Gynecology*		*General surgery (% of total)*
	No.	*% of total*	
Neoplasm, extrinsic	38	44.7*	16.8
Inflammatory strictures	25	29.4†	4.6
Fecal impaction	8	9.4	3.8
Neoplasm, intrinsic	7	8.2‡	44.0
Inflammatory adhesions	2	2.4§	5.3
Operative complications	2	2.4‖	9.2
Volvulus	1	1.2	8.4
Postoperative adhesions	1	1.2	5.3
Miscellaneous	1	1.2¶	3.1

*Ovarian neoplasm 37.7% (malignant 36.5%, benign 1.2%), endometrial carcinoma 3.5%, cervical carcinoma 2.4%, and fallopian tube carcinoma 1.2%.

†Radiation therapy 24.7%, pelvic inflammatory disease and pelvic abscess 3.5%, and diverticulitis 1.2%.

‡All colon carcinoma.

§Radiation therapy 1.2%, pelvic abscess 1.2%.

‖Inadequate end-to-end anastomosis after partial colon resection.

¶Endometriosis.

Results

Incidence of obstruction. During a 25-year period from 1960 to 1984, a total of 368 patients were hospitalized for acute mechanical intestinal obstruction. Forty-six patients (12.5%) had more than one episode of obstruction and were hospitalized two to four times (mean 2.4 times). The 368 patients with intestinal obstruction constitute 1.1% of all patients hospitalized for gynecologic disease. Three hundred five patients (82.9%) had gynecologic cancer or a history of treatment for gynecologic cancer and account for 3.3% of all patients in the oncology section of the gynecology service. Sixty-three patients (17.1%) had benign gynecologic disease accounting for 0.2% of all patients admitted to the benign gynecology section of the gynecology service.

Obstruction of the small intestines was more common than obstruction of the large intestines (283 cases, 76.9% versus 85 cases, 23.1%).

Cause of obstruction. Common causes of obstruction of the small and large bowel are shown in Tables I and II. In Tables I and II the incidence of various causes of obstruction found in patients hospitalized in the gynecology service of the Medical College of Virginia during the study period is compared with the causes of obstruction in a large series of patients in a general surgical ward.[5]

Large bowel obstructions among patients with gynecologic disorders were mostly caused by extrinsic compression from ovarian carcinoma (Table II). An intrinsic process (colon carcinoma) was the single most common cause of large bowel obstruction on a general surgical ward but was also the cause of bowel obstruction of seven patients included in the present series.

Table III. Management of patients with small bowel obstruction by gastrointestinal intubation

Causes of obstruction	*Patients*	*Obstructive episodes*	*GII**	*Obstruction resolved*	
				No.	*% of GII*
Neoplasm, extrinsic	175	219	188	20	10.6
Postoperative adhesions	41	43	43	35	81.4
Inflammatory strictures	34	42	40	8	20.0
Inflammatory adhesions	23	25	24	13	54.2
Miscellaneous	10	10	8	0	0
Total	283	339	303	76	25.1

*GII = Gastrointestinal intubation.

Table IV. Operative mortality rates in patients with intestinal obstruction

Causes of obstruction	*Small bowel*		*Large bowel*	
	Surgical treatment (No.)	*Operative deaths (No./%)*	*Surgical treatment (No.)*	*Operative deaths (No./%)*
Neoplasm, extrinsic	199	45/22.6	40	5/12.5
Postoperative adhesions	8	0	1	0
Inflammatory strictures	34	4/11.8	26	2/7.7
Inflammatory adhesions	12	1/8.3	2	0
Neoplasm, intrinsic	0	0	7	1/14.3
Miscellaneous	10	0	4	0
Total	263	50/19.0	80	8/10.0

These patients developed obstruction of the large bowel while undergoing chemotherapy for presumed ovarian carcinoma. The intrinsic growth pattern of the colonic lesions and identical histologic appearance of the ovarian and colon tumors suggest that in five cases the ovarian malignancies were metastases from previously unrecognized colon carcinomas. In two cases, histologic examination of the ovarian and colon masses showed differences in appearance. It is likely that these patients had two separate cancers at the same time. Only one of the seven patients underwent a barium enema during the original workup of the pelvic masses that, in retrospect, showed a defect in the ascending colon. The lesion was misinterpreted as a "spastic stricture."

Management of obstruction by gastrointestinal intubation. The 283 patients with obstruction of the small intestines developed 339 episodes of obstruction requiring separate hospital admissions. In 303 instances, nasogastric tubes or long gastrointestinal tubes (usually Miller-Abbott tubes) were used at the discretion of the attending physician. The results of conservative management with tube suctioning for gastrointestinal decompression are shown in Table III.

In 76 patients (22.4% of all obstructive episodes; 25.1% of patients with gastrointestinal intubation) the obstruction was successfully relieved by tube suctioning, and surgery was avoided. In 122 instances (40.3% of all cases with tube suctioning) patients responded favorably to gastrointestinal intubation, according to clinical and radiographic evidence. However, the patients were unable to tolerate oral feedings (80 obstructive episodes) or were subjected to surgery without the challenge of oral feedings (42 obstructive episodes). The latter group consisted mainly of patients who were previously treated conservatively for bowel obstruction or for whom surgery was required for conditions such as bulky neoplasms, hernias, or endometriosis.

In 105 instances (34.6% of all patients subjected to tube suctioning) gastrointestinal intubation did not result in clinical or radiographic improvement. All 227 patients with obstructions not relieved by tube suctioning were treated surgically. Thirty-six additional patients underwent surgery within 6 hours after the diagnosis of small bowel obstruction was made, without having undergone a trial of intestinal decompression with tube suctioning.

Seventy-seven patients with colon obstruction not caused by fecal impaction had 80 instances of obstruction. Gastrointestinal intubation was used during 60 of these obstructive episodes (75%) in preparation for surgery. The eight patients with fecal impaction were treated conservatively by enemas or ingestion of laxatives and mineral oil.

Management of obstruction by surgery. In the 263 episodes of small bowel obstruction managed by exploratory laparotomy, corrective surgery consisting of lysis of adhesions, resection of tumor compressing the bowel, or resection of the obstructed bowel segment and reanastomosis of the free bowel ends was possible

in 189 cases (71.9% of all operations). In 50 cases (19%) the obstructive bowel segment was bypassed and a portion of the bypassed bowel was exteriorized as a mucous fistula proximal to the obstruction. In 24 (9.1%) of the patients who underwent exploration, corrective surgical treatment could not be carried out because of carcinomatosis.

Surgery was performed in all 80 cases of large bowel obstruction not caused by fecal impaction. Primary resection of the obstructing lesions or the obstructed bowel segment, with or without reanastomosis of the colon, was possible in 43 cases. In 24 of the 43 cases the proximal colon was decompressed by a (usually transverse) colostomy, which was reversed at a later time in 16 cases. In 19 cases the obstruction was relieved without the need for colostomy. In 37 obstructive episodes, fecal diversion was carried out by means of colostomy (32 cases), ileostomy (four cases), and cecostomy (one case). The obstructing lesion or the obstructed colon segment was resected and bowel continuity reestablished later in 16 (43.2%) of these patients.

Deaths. Patients who were successfully treated for small bowel obstruction by tube suctioning alone had mostly postoperative adhesions or adhesions related to radiation therapy. In the absence of active cancer, none of these patients died of sequelae related to bowel obstruction.

The prognosis of surgically treated small bowel obstruction depended on the underlying cause of the obstruction. The mortality rate was the highest for patients with malignant neoplasms and radiation damage of the bowel (inflammatory strictures and adhesions) (Table IV). Twenty-four patients whose obstruction could not be relieved surgically died of complications related to the persistent obstruction. Four patients with presumed surgical relief of the obstruction had persistent obstruction or developed recurrent obstruction leading to death within 30 days of the surgery. Other causes of postoperative death include peritonitis (10 cases), sepsis (four cases), pulmonary embolus (three cases), cerebrovascular accident (two cases), myocardial infarction (two cases), and pneumonia (one case).

Eight patients with obstruction of the large intestines suffered fatal complications (Table IV). Their deaths were caused by peritonitis (three cases), sepsis (two cases), persistent obstruction as a result of carcinomatosis (two cases), and pulmonary embolism (one case).

Comment

Incidence of obstruction. Obstruction of the large and small intestines in gynecology is most commonly (83%) associated with pelvic neoplasms, most notably ovarian carcinoma. Almost 50% of the patients who die of ovarian cancer can be expected to develop small bowel obstruction during the illness.[6] More than 3% of patients on the gynecologic oncology service of the Medical College of Virginia had bowel obstruction. By contrast, patients admitted for benign gynecologic disease had bowel obstruction in 0.2% of the cases. Obstruction of the small intestines was more common than obstruction of the large intestines (77% versus 23%).

Causes of obstruction. The most common causes of small bowel obstruction associated with gynecologic disease were extrinsic compression from ovarian carcinoma, postoperative adhesions and inflammatory strictures, and adhesions secondary to radiation therapy. By contrast, patients on a general surgical ward were more likely to have postoperative adhesions and external hernias causing obstruction of the small intestines. More than 20% of all small bowel obstructions caused by adhesions result from gynecologic surgery.[7]

Obstruction of the large bowel in patients with gynecologic disorders was caused mostly by extrinsic compression from ovarian carcinomas, inflammatory strictures secondary to radiation therapy, and fecal impaction. Although much more common on a general surgery ward, colon carcinoma was responsible for large bowel obstruction in seven patients in this series who were treated for ovarian carcinoma. This fact underscores the need for careful evaluation of the large bowel by testing for occult blood and performing appropriate endoscopic or roentgenographic studies in patients thought to have ovarian carcinoma. Thorough intraoperative palpation of the entire large bowel should always be part of an abdominal exploration.

Management of obstruction by gastrointestinal intubation. Gastrointestinal intubation is highly effective for treating small bowel obstruction caused by postoperative adhesions. The success rate of 81% in the present series is similar to those reported by other authors.[8,9] Partial small bowel obstruction is more likely to respond to tube suctioning than is complete obstruction.[8-10] Obstruction occurring <30 days after surgery has a better prognosis than obstruction occurring >30 days after surgery.[9] More than 70% of all small bowel obstructions caused by adhesions will respond to intestinal suctioning within 24 hours.[10]

Tube suctioning is rarely successful when the obstruction is caused by neoplasms or strictures associated with radiation. Patients who do respond to gastrointestinal intubation may be managed temporarily by dietary modifications consisting of a low-residue diet or dietary supplements with little or no residue. However, the anatomic defect tends to worsen, and surgery eventually becomes necessary in nearly all patients.[6] Only occasionally will a patient with bowel obstruction secondary to ovarian cancer respond to chemotherapy and have long-lasting benefits from conservative management.[11]

Traditionally, long gastrointestinal tubes such as the Miller-Abbott tube, Baker tube, or Cantor tube were employed whenever possible to achieve maximum decompression of the distended bowel proximal to the obstruction. Although occasionally time-consuming and laborious, passage of long tubes into the small bowel can be achieved in one attempt in 70% of patients with obstruction as long as bowel peristalsis is present.[8] Brolin[10] recently suggested that partial small bowel obstruction may be treated more efficiently with a nasogastric tube instead of a long tube. In cases of complete small bowel obstruction requiring surgical intervention the advantages of a long tube outweigh its disadvantages. Particularly in cases of obstruction caused by adhesions, intraoperative advancement of a long tube through the entire bowel serves to "splint" the intestinal loops and may prevent recurrence of the obstruction. In addition, intestinal distention may be relieved by suctioning bowel contents intermittently while the tube is being advanced intraoperatively. Although this maneuver facilitates closure of the abdomen, it may also retard resumption of bowel activity.[12]

Treatment of large bowel obstruction is primarily surgical, and decompression of a severely distended colon is achieved by temporary colostomy of the transverse colon. Gastrointestinal intubation for removal of swallowed air and succus entericus may still be of value while the patient is being prepared for surgery. Unless there is distention of the small bowel as a result of incompetence of the ileocecal valve, use of a nasogastric tube appears to be appropriate.

Management of obstruction by surgery. Although early surgical intervention within 12 to 24 hours after diagnosis of the obstruction has been advocated,[7, 13] the more recent surgical literature suggests that an expectant behavior of up to 48 hours and even longer appears justified as long as serial x-ray films and clinical evaluation indicate improvement of the intestinal distention.[6, 10] Lack of improvement or worsening of the clinical and radiographic findings beyond 12 to 24 hours indicates a high complication rate and poor prognosis.[5, 6, 14]

The surgical treatment of small bowel obstruction is dictated by the underlying cause of the problem. Whereas lysis of adhesions and splinting of the small bowel is appropriate for treating obstruction caused by adhesions, obstruction caused by tumors and strictures is best managed by resection of the obstructed bowel segment and reanastomosis of the free bowel ends. In 19% of the patients in the current study, this approach was not feasible because of extensive tumors. The obstructed bowel segment is then bypassed and a suitable portion of the bypassed bowel is exteriorized as a mucous fistula proximal to the obstruction. In some 9% of the patients the entire small bowel may be encased by tumor or may form a conglomerate with the tumor so that surgical relief of the obstruction is not possible.

Treatment of large bowel obstruction by primary resection is feasible when the degree of intestinal distention is not severe. In almost half of the patients studied, severe distention of the colon required a fecal diversion by colostomy, ileostomy, or cecostomy. Advanced cancer or changes associated with radiation prevented bowel continuity from being reestablished in most cases.

Operative deaths. The operative mortality rate of bowel obstruction associated with gynecologic disease is high and reflects the underlying cause of the disease. Most patients who died during the postoperative period had advanced ovarian carcinoma. The prognosis of these patients is quite unpredictable by comprehensive assessment of risk factors.[3] The prognosis is poor for patients with rapidly reaccumulating ascites, advanced age, severe nutritional deprivation, liver metastases, or distant metastases or those who have responded poorly to combination chemotherapy or total abdominal radiation therapy.[3]

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. James L. Purcell, Charleston, South Carolina (Official Guest). This is an excellent review of 368 gynecologic patients with intestinal obstruction at the Medical College of Virginia between 1961 and 1984. Drs. Krebs and Goplerud's primary goal was to establish and clarify guidelines for gynecologic surgeons in the management of intestinal obstruction. They also contrasted etiologic differences of intestinal obstruction in the general surgery and gynecologic services. They indicated that it is the cause of the obstruction in the gynecologic patient that can be used as one of the primary tools in determining the sequence and modality of management, as well as predicting postoperative morbidity and mortality rates.

In this review intestinal obstruction accounted for only 1.17% of the gynecologic admissions. Although the incidence is not extremely high, this is a very dramatic complication for both the patient and her physician.

It is of interest that 83% of the patients had some type of gynecologic malignancy as the cause of obstruction. The vast majority of patients had ovarian carcinoma. Dr. Krebs stated that 50% of the patients who die of ovarian carcinoma have an intestinal obstruction some time during the course of the disease. The remaining 17% are most likely to be of concern to the primary gynecologist. These include radiation, strictures and adhesions, postoperative adhesions, and inflammatory strictures and adhesions.

Small bowel obstructions were far more common than those of the large bowel: 77% and 23%, respectively. These figures compare favorably with those of other teaching institutions.

Three hundred three of 339 episodes of small bowel obstructions were treated initially with gastrointestinal intubation. As other authors have confirmed, this is highly effective for small bowel obstruction secondary to postoperative adhesions. In this review small bowel obstructions that occurred within 30 days of the primary surgery had a better prognosis than those that occurred after 30 days. The small bowel obstructions secondary to malignancy or radiation were rarely totally responsive to gastrointestinal intubation. Large bowel obstruction virtually never responded to intubation even with the long tube. Their review also indicated that if an obstruction is going to resolve with intubation it will do so within 24 hours. The older literature dictated that morbidity and mortality rates increased with expectant management after 24 hours. However, Krebs and Goplerud's review indicated that as long as there was clinical and radiologic improvement, the risk did not increase with expectant management up to 48 hours or longer, although with failure to improve or deterioration of the patient's condition, surgical intervention was indicated.

The surgical management of small bowel obstructions constituted lysis of adhesions if indicated. If the obstruction was secondary to malignancy, resection of the involved bowel and tumor or bypass procedures produced the best result. In 9% of the obstructions in this review the small bowel was totally encased with tumors and resection was impossible.

The 80 large bowel obstructions were surgically treated by primary resection with or without reanastomosis or colostomy and resection at a later date. It was of interest that five of the larger bowel obstructions were primarily felt to be secondary to ovarian malignancy but at surgery were found to be primary colon carcinoma with metastasis to the ovary. Two patients had double primary lesions of the colon and ovary. Drs. Krebs and Goplerud stressed that the bowel must be thoroughly inspected for other malignancies at the time of surgery.

Drs. Krebs and Goplerud suggested in this review that one of the long gastrointestinal suction tubes should be used in all cases of complete obstruction where decompression is needed. This is in contrast to some articles in the current literature and my discussion of the matter with other gynecologic oncologists. Their opinion was that the nasogastric tube was adequate for intestinal obstruction secondary to postoperative adhesions or inflammatory adhesions. Obstruction secondary to radiation or malignancy may respond to a nasogastric tube, but if not, a long tube may be substituted.

I feel that Drs. Krebs and Goplerud have accomplished their goals in this review by giving us a better insight into intestinal obstruction as it relates to gynecologic disease. Their comparison of the etiologic factors and their prognostic value for gynecologic intestinal obstruction and general surgical intestinal obstruction were helpful. The care of most gynecologic malignancies is referred to oncologists today and therefore they see the vast majority of intestinal obstructions.

In a paper Dr. Krebs presented at this meeting last year, he made a plea for better training in gastrointestinal surgery for our residents. I concur with this in every respect. However, with the cloud of litigation hanging over our heads, the private gynecologists may save himself questions later if he obtains a surgical consultation to assist in the management of bowel obstruction. I would like to hear Dr. Krebs' feelings about this aspect of the management of bowel obstruction.

Dr. Thomas K. Young, Gainesville, Florida. I cannot help but think that the majority of benign or obstructive complications secondary to adhesions would end up in general surgical literature. I wonder if Drs. Krebs and Goplerud have some information as to what percent of adhesive obstruction in general surgical series is related to initial gynecologic surgery.

Dr. Matthew Burrell, Atlanta, Georgia. I would like to know whether bypass or resection was used in the group who underwent irradiation. There is information to suggest that resection carries a higher morbidity rate.

Dr. Krebs (Closing). Dr. Purcell made a comment regarding the use of long tubes in all cases. I think the way the article should have read is that the long tube was used in the majority of the cases. There were certainly patients there who were treated with nasogastric suction. Our prime cause for using the nasogastric suction tube placed into the stomach would be in patients in whom there are no bowel sounds or function evident on ascultation. In those cases you cannot expect that a long tube will be transported to where it is needed. In the stomach a long tube is not as effective as the standard nasogastric tubes. A second point would be in regard to the management of the patients who have bowel problems related to general gynecologic problems. The figures that I showed you indicate quite clearly that the general gynecologist is not going to encounter many cases of bowel obstruction associated with the diseases he treats. Nor is he generally capable of handling relatively complicated procedures related to bowel obstruction, which brings me to the comment that those patients will probably be operated on in association with a gynecologic oncologist or would simply be referred to a general surgeon. The final comment on bypass versus resection is an issue that is still being debated by gynecologic oncologists. I prefer resection of the lesion if this can be done with reasonable effort. In many of the patients the effort is unreasonable and it is a much simpler procedure to bypass the obstructed, usually heavily irradiated bowel that is stuck together deep in the pelvis. However, we have noticed that the outcome has been quite favorable in those patients in whom a bowel resection in a radiated patient was attempted and carried out. Once again it lies somewhat in the individual's judgment and his surgical experience which of the two methods of surgery he chooses.

Fetal death: Diagnosis and management

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Death of the fetus after 20 weeks of gestation complicates about 1% of pregnancies. Of various means of diagnosing fetal life and death, real-time ultrasound visualization of the fetal heart is the most accurate. Delivery of the dead fetus can be effected by various means, but in most instances, at least before 28 weeks and perhaps thereafter as well, the simplest and most effective method is with prostaglandin vaginal tablets. A variety of conditions are known to cause fetal death or increase the risk that it will happen, but these account for only about 50% of cases. Four special tests may identify a cause of the "unexplained stillbirth" in the other 50% of cases. These tests include karyotype, listerial culture, fetomaternal hemorrhage, and lupus anticoagulant. (Am J Obstet Gynecol 1987;157:583-9.)

Key words: Fetus, death, causes, diagnosis, therapeutic options

The death of a fetus is one of the unhappy events in the field of obstetrics. A particularly distressing experience that is encountered all too often is its occurrence utterly without warning in a pregnancy that has previously seemed entirely normal. Though certain diseases and conditions in the mother or the fetus are associated with increased risk, much of the time the cause of fetal death cannot be identified by even the most thorough search, intensifying the sense of frustration felt by both patient and physician.

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The definition of fetal death, specifically the stage of gestation encompassed by the definition, differs among various public health authorities, which makes comparison of fetal mortality problematic.[1] The World Health Organization defines fetal death as that occurring before the complete expulsion or extraction from the mother of a product of conception, irrespective of the gestational age. However, the U.S. National Center for Health Statistics uses only deaths at or after 20 weeks of gestation to report vital statistics. To complicate the situation even further, six states (Colorado, Georgia, Hawaii, New York, Rhode Island, and Virginia) require the report of a fetal death at any point of gestation, and at least some of the time they calculate their state rates on the basis of all reported deaths. Finally, certain countries other than the United States, particularly developing nations, calculate fetal mortal-

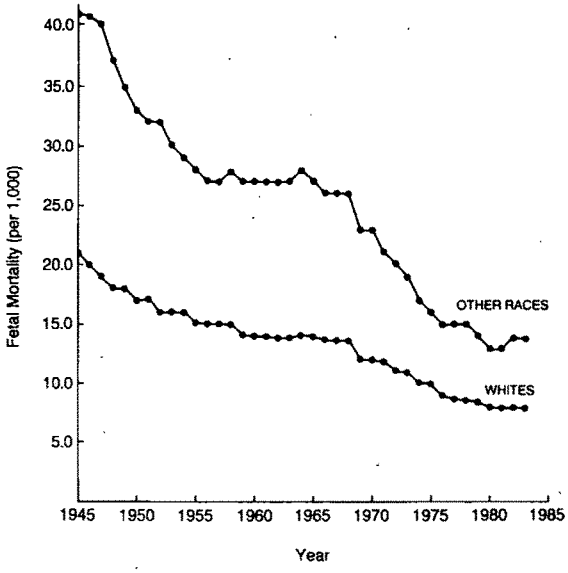


Fig. 1. Fetal mortality reported by National Center for Health Statistics. [Reproduced with permission from *Clinical Obstetrics and Gynecology.* Petitti D. Clin Obstet Gynecol 1987;30:253-8.]

ity on fetal deaths at ≥28 weeks of gestation. Thus, whenever a fetal mortality is mentioned, it is essential to know the definition of fetal death that is used to arrive at the numerator. It is also important to know whether the denominator represents total or live births. Technically, the use of the latter provides a "ratio" rather than a "rate."

Fetal death rates over the last 40 years in the United States are illustrated in Fig. 1. The most striking features is the racial difference, which although it has narrowed progressively, continues to indicate a substantial advantage of whites over numbers of other races. Another features of this illustration of fetal mortality patterns is the relative stability in both races during the decade of the 1960s. Many changes in care practices—family planning, abortion, maternal nutrition, fetal monitoring, and neonatal intensive care, to mention only a few—have been claimed as responsible for the decline in fetal mortality since 1969. However, the complexities of the relationships do not permit estimation of the relative contribution of any of these factors.

Causes

Only relatively infrequently is it possible to identify a specific condition that directly causes death of the fetus. Umbilical cord prolapse and premature separation of the placenta are examples of such direct causes. More often conditions are known by experience to be associated with increased risk of fetal death, though the precise mechanism is obscure. Among these types of associated conditions are various medical complications

Table I. Causes of fetal death

Cause of death	*Percent of deaths*
Hypertensive disease	5-25
Medical complications	5-10
Erythroblastosis fetalis	3-15
Cord and placental complications	10-20
Congenital malformation	5-10
Intrauterine infection	5-15
Unexplained	~50

of pregnancy, notably hypertensive disease and diabetes mellitus. Though many controversies and uncertainties surround the topic of postdatism, there can be little doubt that the risk of fetal death increases as gestation extends beyond 42 weeks.

Table I lists various causes of fetal death, along with an estimate of the proportion of deaths associated with each, drawn from a variety of sources in the literature and from personal experience. Ranges are given, since the proportions vary considerably in different populations. For example, hypertensive disease (usually acute but occasionally chronic) accounts for as few as 10% of fetal deaths in some series but as many as 25% in others, depending on the frequency of the disease in the particular population from which the series is drawn. These data also vary with the era from which they come. For example, erythroblastosis fetalis caused by rhesus factor isoimmunization formerly accounted for 15% to 20% of fetal deaths in some series, but the proportion has declined, both relatively and absolutely, with the advent of rhesus factor immunoprophylaxis. However, it is important to note that the problem of rhesus factor sensitization has not gone away altogether.

The most striking feature of the cause of fetal death is that when all the known and suspected causes and associated conditions are added together, they account for no more than 50% of observed fetal deaths, leaving half or more undiagnosed and presumably undiagnosable as far as causation is concerned. Every physician who practices obstetrics for any period of time has had a distressing experience something like the following: some time during late gestation, a woman who has had an entirely normal pregnancy reports with a certain anxiety in her voice that she has not felt her fetus move for some hours or 1 or 2 days. Auscultation fails to identify a fetal heart beat, and real-time ultrasound confirms the absence of cardiac activity in the fetus.

This scenario, all too familiar to every obstetrician, is distressing to the physician and devastating to the patient. Typically, it prompts a frantic and haphazard search to determine the cause, a search which most of the time proves fruitless as far as any specific findings are concerned.

Autopsy of the stillborn fetus typically consists of a cursory gross examination that leads to a report of "macerated stillborn." Thus, at least as customarily conducted, the autopsy adds little in resolving concerns about causation. However, a careful and meticulous examination by an individual experienced and skilled in perinatal pathology can illuminate a substantial proportion of the ≥50% of cases for which no cause is otherwise apparent.[2,3] If an autopsy is not to be done, a full-body radiograph of the fetus can identify skeletal abnormalities.

Diagnosis

Suggestive signs of fetal death, specifically the woman's inability to perceive fetal movements and the physician's inability to hear the fetal heart beat, are imprecise at best. Before acting it is necessary to establish the diagnosis with absolute certainty, something that is rarely easy.

Radiography. For more than 50 years, radiography was the principal means of confirming the diagnosis of fetal death. The most reliable sign, the presence of gas in the fetal vasculature (Fig. 2), is also a useful test, since it can appear within 1 or 2 days of death. Its principal disadvantage lies in its low sensitivity; no more than half of fetal deaths are accompanied by this sign.

Other radiographic signs reflect one or another manifestation of fetal maceration and are less specific. Moreover, these are of less use clinically because of the time required (10 to 14 days) for their development. Overlapping cranial sutures, known as Spalding's sign, develop eventually in nearly all cases but may be mimicked by the normal molding process that accompanies labor. Abnormal angulation of the fetal spine as a result of collapse of the vertebral bodies, a quantitative change, is sometimes difficult to recognize.

Ultrasound. Ultrasound imaging of fetal cardiac activity has, over the past decade, become the most useful diagnostic test in cases of suspected intrauterine death. A-mode or time-motion ultrasonography may be used, but the simplest and most useful technique is that which uses real-time equipment. Heart wall motion and valvular function can be visualized readily, and failure to detect this activity after 10 to 12 menstrual weeks of gestation constitutes reliable evidence of fetal death.[4] However, the ultrasound diagnosis of fetal death is somewhat more difficult than that of fetal life; it should be confirmed by two independent observers, each taking a minimum of 3 minutes of study.[5]

Though real-time ultrasound has become the "gold standard" for antenatal diagnosis of fetal life or death, it should be remembered that this technique has a subjective component that makes it liable to observer error. In one case at the University of Iowa, a woman suspected of having intrauterine fetal death was examined by an experienced sonographer who confirmed the diagnosis, only to deliver a living infant several days later.



Fig. 2. Abdominal radiograph taken 2 to 3 days after fetal death in late pregnancy. *Arrows* indicate gas in fetal aorta and/or vena cava.

Other ultrasound findings with fetal death include scalp edema and other maceration sequelae that can be visualized radiographically. Occasionally, these adjunctive findings are useful.

Amniotic fluid and hormones. The amniotic usually becomes discolored at about the same time the fetus dies. Most often it is a deep mahogany-red shade, the so-called "tobacco juice" appearance that apparently results from hemoglobin products or the thick brown "pea soup" that characteristically reflects the passage of meconium. However, these changes are neither entirely sensitive nor entirely specific and should be regarded as adjunctive or confirmatory signs. The volume of amniotic fluid is often diminished in cases of fetal death, but whether this precedes or follows the event is unknown.

The concentration of the enzyme creatine kinase in amniotic fluid increases rapidly with fetal death, from <3 σ U/ml in normal circumstances to >5 σ U/ml 24 to 48 hours after death.[6] This test may have some use in occasional instances.

Among the various pregnancy hormones, estriol is

the most useful index of fetal health or disease. Levels in either blood or urine decrease dramatically 24 to 48 hours after fetal death, a finding that can be useful diagnostically. Progesterone is less helpful because, as a hormone entirely of placental origin, production may be maintained for variable times after the fetus dies. Chorionic gonadotropin levels may have some value in assessing early pregnancy complications, including embryonic and fetal death. α-Fetoprotein levels in maternal serum and amniotic fluid are elevated with fetal death, but the finding is not specific enough for use in diagnosis.

Management

Once the diagnosis of fetal death is established, the proper management needs to be decided on. However, some appreciation of the natural history is essential in evaluating the therapeutic options. At least 75% of women labor spontaneously within 2 weeks of fetal death, and most of the others will have delivered by the time another 2 weeks have elapsed. However, some variation in this timetable exists with respect to the timing and the cause of fetal death. As a general rule, the interval between death and spontaneous labor varies inversely with the stage of gestation at which the fetus dies. In other words, the earlier in pregnancy the death occurs, the longer the period of latency until the fetus is expelled. Moreover, certain causative conditions seem to be associated with longer latency periods than others for reasons that are not altogether clear. A case in point is erythroblastosis fetalis, which is well-known to predispose to prolonged retention of the dead fetus.

The development of a coagulopathy associated with the dead fetus syndrome was first recognized >30 years ago. Initially thought to result from erythroblastosis fetalis (a misconception that can be understood in view of the commonness of rhesus factor isoimmunization in that era and the tendency for prolonged retention of the fetus that died from erythroblastosis), it is now recognized as a potential complication of fetal death from any cause. The pathophysiology involves release of thromboplastic substances from the products of conception into the maternal circulation, promoting disseminated intravascular coagulation that gradually consumes the maternal clotting factors, particularly fibrinogen and platelets.

Development of the consumptive coagulopathy that accompanies fetal death is a gradual process, in contrast to that accompanying abruptio placentae or amniotic fluid embolus. Laboratory evidence does not appear until 3 or 4 weeks after the fetus dies, and thereafter the serum fibrinogen level and/or platelet count decrease progressively but slowly; the rate of decline in fibrinogen level has been quantitated at 20 to 85 mg/dl/week.[7] Thus clinical hemostasis is maintained for some time. In expectant management of a woman who has sustained fetal death, it is not necessary to measure coagulation indices until several weeks have elapsed, and then a sampling interval of once weekly generally provides adequate monitoring. Hemorrhage from coagulopathy will not occur until the coagulation indices reach critical levels, such as a serum fibrinogen value <100 mg/dl and a platelet count <50,000/mm^3, which rarely if ever occurs before 6 weeks from the time of death. Sampling once or twice weekly is ample to identify those patients who need intervention (i.e., delivery) before hemorrhage becomes a threat.

Therapeutic options. At least five options are available to the clinician caring for a woman whose fetus has died: (1) expectancy; (2) mechanical evacuation through the cervix; (3) intravenous administration of oxytocin; (4) amnioinfusion with hypertonic solution; (5) prostaglandin administered vaginally, systemically, or intra-amniotically. Each offers both advantages and disadvantages, and none is ideal in every case.

Expectant or conservative management represents a reasonable choice in that spontaneous expulsion will occur eventually in all cases. Moreover, it is safe as long as the maternal coagulation status is monitored as outlined previously and changes approaching the danger zone are acted on by effecting prompt delivery. The disadvantage of the conservative approach lies in its adverse emotional impact. Fetal death, particularly when it occurs unexpectedly but also when risk is known to be high beforehand, is emotionally devastating and the near-universal patient response is an overwhelming desire to have the uterus emptied as soon as possible. The prospect of waiting days and even weeks for spontaneous labor is simply unacceptable to the overwhelming majority of women. Nevertheless, it may be the treatment of choice in the occasional instance in which coexistent medical conditions contraindicate other therapeutic options.

Mechanical dilation of the cervix and evacuation of the uterus by suction or other means is clearly the method of choice when the uterus is small enough to permit safe performance of the operation. What uterine size is regarded as "safe" will vary with the physician's skill and experience with this type of surgery. For most gynecologists, the point at which risk-benefit analysis argues against this approach lies somewhere in the junction between the first and second trimesters (i.e., uterine size of 13 to 14 weeks of gestation). In any case, transcervical evacuation of the missed abortion–dead fetus carries increased risk of bleeding, so the operation should always be approached with caution. Specifically, it should be done in a properly equipped surgical facility (rather than an ordinary clinic), an intravenous infusion must be initiated, and blood should be available.

Oxytocin given in dilute intravenous infusion offers advantages by virtue of its familiarity and relative safety. However, although it typically promotes uterine contractions, its efficacy in effecting delivery may be limited, particularly in cases of fetal death remote from term. Large doses and prolonged administration may be required, circumstances that increase the risk of water intoxication and attendant central nervous system complications. To avoid such very serious sequelae, extreme care to avoid prolonged administration in large volumes of electrolyte-free solutions must be exercised.

Amnioinfusion with a hypertonic solution, most commonly sodium chloride but occasionally glucose or urea, is effective in emptying the uterus. Its principal disadvantage involves the small but definite risk of serious complications. Because of the oligohydramnios that quite regularly accompanies fetal death, amniocentesis can be technically difficult in such cases, and there may be uncertainty as to the exact location of the needle. If the tip of the needle should lie outside the uterine cavity (e.g., in the uterine wall or a maternal blood vessel), rapid infusion of a large volume of hypertonic solution can be fatal. Another risk, at least theoretically, is that of aggravation of any concomitant coagulopathy. For these reasons, amnioinfusion has a limited value in the termination of pregnancies in which the fetus has died.

Prostaglandins (PG) are potent uterine stimulants, and a number of different PG types and formulations have been used in effecting delivery of the dead fetus. The most commonly used approach involves PGE_2, administered intravaginally and available commercially as suppositories containing 20 mg of the active agent. Typically given as one suppository and repeated at 3- to 4-hour intervals until delivery is imminent (usually within 12 hours), this treatment has been demonstrated to be highly effective in a number of studies.[8] Side effects are frequent, nearly all patients exhibit some degree of fever and tachycardia and half or more have vomiting and/or diarrhea, but these typically present only minor problems clinically. Moreover, they may be ameliorated by lowering the dosage (e.g., to half of a suppository), lengthening the interval, and pretreating with medications for the various side effects. An uncertainty about intravaginal PGE_2 involves the product-labeling injunction that it be used only in deaths occurring up to 28 weeks of gestation, presumably reflecting rarely reported serious complications, particularly uterine rupture[9] in later pregnancy. Though several reports have documented safety with third-trimester usage,[10] the matter of the labeling statement makes its use under such circumstances problematic. For example, if a patient is given PGE_2 vaginal tablets in late pregnancy and sustains a complication of any type and for any cause, the responsible physician may be in jeopardy (or at least be in an uncertain situation) in a medicolegal sense. If use in late pregnancy is contemplated, lowering the dose and avoiding concomitant oxytocin use (since this has been employed in the serious complications reported) seem prudent.

Other PG formulations and routes of administration have been used in managing fetal death. Intravenous PGE_2 (0.5 to 2.5 μg/min), intramuscular 15-methyl $PGF_{2\alpha}$ (250 mg every 2 hours), and intra-amniotic $PGE_{2\alpha}$ (40 mg) have all been reported effective in relatively small series.[8] In general, both efficacy and side effects seem similar to those related to the more familiar PGE_2 vaginal suppositories.

Management suggestions. In view of the variety of options available and their advantages and disadvantages, individualization of treatment should be regarded as the cornerstone of management. However, it is possible to set forth certain guidelines.

The instance in which uterine size permits the prospect of reasonably safe cervical dilation and evacuation by instruments and/or suction is straightforward. The risk of hemorrhage is greater than that with interruption of normal pregnancy, and as indicated previously, special precautions in this regard are indicated.

Among cases uncomplicated by other diseases or conditions, fetal death during the second trimester can usually be managed most simply and effectively by PGE_2 vaginal tablets, perhaps with premedication to minimize side effects. When death has occurred during the early third trimester (e.g., between 28 and 34 weeks), amnioinfusion with hypertonic saline solution may be considered if technical conditions (including real-time ultrasound monitoring) provide assurance of safety; intravaginal PGE_2 (in reduced dosages) also represents a viable option if the physician is comfortable with the medicolegal uncertainties involved. With fetal death in the last month or 6 weeks of gestation, intravenously administered oxytocin is probably the best choice; an expectant approach may also be considered, since the period of latency is likely to be short. In all cases, osmotic dilators of the cervix may be useful adjuncts.

The presence of coexisting complications may require some modifications in these gudielines. For example, PG usage in any form is likely to be accompanied by cardiovascular side effects, making these agents at least relatively contraindicated in women with significant cardiovascular or renal disease. Amnioinfusion of hypertonic saline solution should probably also be avoided with these medical complications, and intravenous fluid volume in oxytocin treatment requires careful attention. In view of the tendency to bronchospasm with PGs, these agents should be used with caution in patients who have asthma.

In the rare case in which a coagulopathy has devel-

oped because of neglect, a course of intravenously administered heparin will restore coagulation factor levels to normal before uterine evacuation.[11]

An unusual but potentially serious complication is the death of one fetus of a multifetal gestation. If monozygotic, the surviving fetus or fetuses may be at some risk of thrombotic complications apparently caused by fetal-fetal exchange of thromboplastic material through vascular shunts in the monochorionic placenta.

Searching for the cause

The occurrence of an unexplained fetal death (i.e., one that happens unexpectedly and is unaccompanied by any apparent cause or associated conditions) prompts an immediate and often somewhat disorganized search for the cause. Typically, the history is gone over in detail, the syphilis serology is repeated, the blood pressure and kidney function are assessed, antibodies to a variety of viruses and other infections are measured in the woman's blood, and a perfunctory autopsy is performed. These results usually fail to reveal anything of significance. The patient is told that she is in that 50% of situations in which there is no known cause, and she is given variable (and sometimes impenetrable) advice about the prognosis in a subsequent pregnancy.

This investigative protocol has become ritualized, and as with any ritual, it has lost any original meaning. However, several "special tests," some new and others not so new, exist, which offer a much higher yield in identifying the cause of a fetal death. Four tests will be discussed briefly: (1) karyotype, (2) listeriosis, (3) fetomaternal hemorrhage, and (4) lupus anticoagulant.

Karyotype. Some 5% to 6% of stillbirths demonstrate some type of chromosomal abnormality,[12] an incidence 10 times that in live births. As a general rule, the earlier in gestation that fetal death occurs, the larger the proportion of chromosomal anomalies. By far the most common karyotypic abnormality is trisomy. Among 2517 spontaneous abortions and fetal deaths up to 28 weeks studied by Warburton et al.,[13] 55% of the abnormal karyotypes were trisomies (most commonly trisomy 16, which never occurs in live births, followed by 21, 22, 13, 15, and 2). The remaining abnormal cases included monosomy X (18%), triploidy (15%), and tetraploidy (6%).

Although karyotyping has a relatively high yield when applied in all cases of fetal death, the substantial cost of chromosome culture makes some selectivity advisable. Among findings associated with chromosomal abnormalities, which therefore can be expected to increase the yield, are (1) physical stigmas that suggest a single gene disorder, (2) multiple anomalies, (3) fetal growth retardation, (4) Potter's syndrome, and (5) nonimmune hydrops.

The success of chromosome culture varies inversely with the time between death and delivery. Nevertheless, the success rate is still reasonable, even when substantial autolysis has taken place, so the study should be used in any case in which it seems to have potential value. The diaphragm is the best tissue to culture, and in cases in which amniocentesis is possible, amniotic fluid cells may be used.

Listeriosis. *Listeria monocytogenes* is a gram-variable rod that causes mild and often asymptomatic infection in the adult but a much more serious condition in the fetus. The principal route is believed to be transplacental from maternal septicemia, leading to an abscess formation in the placenta and then to fetal septicemia. More rarely, infection may occur by an ascending route to cause chorioamnionitis.

Several outbreaks of listeriosis have occurred in recent years, principally from ingesting contaminated foods, especially cheese and other dairy products. Reports of these have included a number of perinatal deaths caused by infection of pregnant women, implying that the fetus is at special risk.[14, 15] The magnitude of any risk is unknown, since no data on listeriosis in nonfetal cases exist.

Because fetal listeriosis can occur with little in the way of maternal illness (at most, a mild and nonspecific influenza-like syndrome), the possibility should be considered in otherwise unexplained stillbirth. The organism grows relatively slowly in culture and may be missed unless sought specifically. Therefore, the clinician should notify the laboratory of the possibility of listeriosis when sending placental or fetal cultures.

The greatest importance of listeriosis lies in its ready response to treatment with ampicillin. If diagnosed and treated early enough, fetal death can be prevented.[16] Even if it is not recognized until after the fetus has died, there exists the potential of treatment in a subsequent gestation.

Fetomaternal hemorrhage. The fact that fetal blood can gain access to the maternal circulation and thereby provoke a maternal immune response has been recognized for >40 years. Only relatively recently, however, has it been appreciated that such fetomaternal hemorrhage can be large enough to cause fetal exsanguination. Various types of trauma can be responsible, but in the majority of instances the pathophysiology is entirely unclear.

Laube and Schauberger[17] studied 29 women with unexplained fetal deaths by Kleihauer-Betke staining of peripheral blood and found four instances of massive fetomaternal hemorrhage, representing 150 to 240 ml of fetal blood (50% to 75% of total volume) in the maternal circulation.

Testing of maternal blood for fetal hemorrhage by either the Kleihauer-Betke technique or any of several other available methods should be done in all cases of

fetal death, particularly when no cause is apparent. Maternal blood must be drawn before induction of labor, amniocentesis, or other manipulation.

The significance of diagnosis of fetomaternal hemorrhage for a subsequent pregnancy is unknown, but it may have implications for immunoprophylaxis in cases of blood type incompatibility. At the very least it can provide an explanation of why the fetus died.

Lupus anticoagulant. Recently, it has become apparent that certain antiphospholipid antibodies are associated with a high frequency of fetal death and other forms of pregnancy loss. The most important of these antibodies is the so-called lupus anticoagulant, a particularly unfortunate term, since its association with lupus erythematosus is imperfect at best and its clinical effects are those of thrombosis rather than of anticoagulation. Closely related is the anticardiolipin antibody that seems to have similar effects.

The manner by which antiphospholipid antibodies affect pregnancy is unclear, but current thinking is that a vasculopathy involving the placenta and/or decidua is involved. In any case, the association with adverse pregnancy outcome is striking, particularly in view of the relatively short time it has been recognized. The most current review, on the basis of 15 retrospective reports published by 1985, documents 242 previous pregnancies in 65 women with lupus anticoagulant, of which 220 (91%) were lost as spontaneous abortion or fetal death.[18] In fact, only six cases have been reported in which a live birth occurred to a woman known to carry the lupus anticoagulant. The timing of fetal losses seems disproportionately high in the second and third trimester.

The screening test for lupus anticoagulant is the activated partial thromboplastin time. If this or other phospholipid-dependent tests show normal results, lupus anticoagulant can be excluded. If the activated partial thromboplastin time is positive (i.e., abnormally prolonged), it should be repeated after mixing with an equal volume of normal plasma to exclude a coagulation factor deficiency. With lupus anticoagulant, the addition of normal plasma will not correct the result to a near clotting time. Thus, if a patient has a prolonged activated partial thromboplastin time, which is unaffected by adding normal plasma, she probably has lupus anticoagulant. More specific tests have been described, but none has yet been used clinically.

The importance of identifying patients with lupus anticoagulant lies in the potential for pharmacologically ameliorating the otherwise abysmal pregnancy outcome. High-dosage glucocorticoid and low-dosage aspirin therapy has been described in relatively small series to promote live births (albeit these births usually are accompanied by preeclampsia, fetal growth retardation, and other complications) in the majority of treated cases.[19]

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Another look at the second-assessment procedure for ovarian epithelial carcinoma

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Over a 10-year period, 65 of 196 patients (33%) with ovarian epithelial malignancy, previously treated with chemotherapy, had a second-look operation if the disease was clinically absent. All procedures were done by one of three gynecologic oncologists, and 48% of the patients had a positive second-look procedure. Of patients with original stage I, II disease, 25% had a positive second-look operation, contrasting with 61% of patients with stage III, IV. Significant perioperative morbidity occurred: one patient had intraoperative vascular collapse; 15% had prolonged ileus; 17% had small bowel resections at the time of the second-look operation or in the postoperative period. Of all patients with negative second-look operations, 24% have had recurrent disease from 5 to 23 months after the procedure. These recurrences were in the liver or distant sites in 63% of the patients. Second-look operations, if still indicated by negative noninvasive techniques, should be performed in tertiary care centers under study situations in a standard fashion. (AM J OBSTET GYNECOL 1987;157:590-6.)

Key words: Second-look, operation, ovarian cancer

The concept of second-look celiotomy for evaluation of presence or absence of disease was initially introduced by Wagensteen et al.[1] in patients who were previously treated for colon cancer. This procedure has become increasingly used during the past 2 decades in the "routine" management of patients with ovarian cancer.[2-10] The application of the term *second-look* procedures has been variable, but most current reports follow the criteria suggested by Ballon et al.[11]: (1) no clinical evidence of persistent carcinoma and (2) no radiographic evidence of persistent carcinoma. Second-look procedures, simply to assess disease status, are rarely advocated for patients with ovarian cancer who were previously treated by irradiation. Complications of the second-look procedure are numerous, with ileus and/or febrile morbidity listed as the most frequent.[2-7, 9, 10] Although laparoscopy, ultrasound, and computed tomography (CT) scanning may detect subclinical, persistent disease in some patients, these modalities are insufficient to assess disease status in most patients who have no clinical evidence of disease. This article reviews the experience at two medical centers in managing patients with epithelial ovarian carcinoma, who met the criteria as candidates for second-look laparotomy.

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Material and methods

The clinical, tumor, and pathology records of all patients with a diagnosis of malignant ovarian tumors at the Medical College of Georgia, Augusta, Georgia, and the Naval Hospital, Portsmouth, Virginia, from January 1975 through December 1984 were reviewed. During the 10-year period, 236 patients were assessed with a diagnosis of ovarian cancer. Of these patients, 40 were initially excluded from the study group, since they had nonepithelial malignant tumors, such as gonadal stromal or germ cell tumors. All patients who underwent second-look procedures met the following criteria: (1) no clinical evidence of disease, (2) no prior radiation therapy, and (3) at least six courses of chemotherapy for known epithelial tumors. Reasons for not performing a second-look laparotomy or for including patients with another operation are noted in Table I. In general, progressive disease was considered a contraindication to second-look laparotomy. All patients who underwent a second-look operation were clinically free from disease on the basis of a thorough physical examination, liver function tests, and chest x-ray films. Most patients had an intravenous pyelogram and barium enema, and in the last 5 years of the study, patients also had pelvic and abdominal CT scans.

All second-look procedures were done under the supervision of one of three gynecologic oncologists (D. G. G., O. E. T., and M. R. D.). Celiotomy techniques

Table I. Reasons second-assessment procedure is not done

Situation	*No. of patients*
Nonresponders to chemotherapy, DOD	64
Tumors of low malignant potential (stage I, II)	7
Postoperative death from original operation	4
Refused chemotherapy, DOD	9
Refused second-look, living	8
Refused second-look, DOD	6
Whole abdominal radiation, alive*	9
Whole abdominal radiation, DOD	5
Dead from chemotherapy Myeloproliferative disorder (3) Cardiac (2)	5
Not "qualified" for second-assessment study†	14
Total	131

DOD = Dead of disease.

*Three patients had stage II, six had stage III disease.

†Ten patients had known tumors before the second-look procedure. Four had a second-look operation done elsewhere.

Table II. Distribution of patients by stage and relationship to positive second-look laparotomy

Stage	*No. of patients*	*Patients with positive second-look (no.)*	*Patients with positive second-look (%)*
I	14	2	14.3
a	6	—	
b	2	1	
c	6	1	
II	10	4	40
a	—		
b	6	1	
c	4	3	
III	37	23	62.2
IV	4	2	50
Totals	65	31	47.7

as described by others[2-10] were used for the second-look procedure. Following a generous midline incision, cell washings from the cul-de-sac, pericolic recesses, and diaphragms were done. Biopsies were done on these peritoneal surfaces and all adhesions. Any remaining greater omentum was removed. Any remaining infundibulopelvic ligaments were resected. Peritoneal surfaces adjacent to the infundibulopelvic and ipsilateral round ligament were also submitted for histologic evaluation. Samples of pelvic and/or para-aortic nodes were obtained in most patients. In the absence of gross tumor, between 15 and 40 tissue samples were submitted

Table III. Grade of tumor and status of second-look laparotomy

Grade of tumor	*No. of patients (%) with positive second-look*	*No. of patients (%) with negative second-look*
Borderline*	4 (44)	5 (56)
1	2 (20)	8 (80)
2	6 (38)	10 (62)
3	19 (63)	11 (37)
Totals	31	34

*All patients had original stage III tumors.

Table IV. Histology of tumor and status of second-look laparotomy

Histologic type of tumor	*No. of patients (%) with positive second-look*	*No. of patients (%) with negative second-look*
Serous	20 (49)	21 (51)
Mucinous	1 (14)	6 (86)
Endometrioid	5 (50)	5 (50)
Adenocarcinoma, unspecified	4 (67)	2 (33)
Malignant brenner	1 (100)	—
Totals	31	34

for each patient. If patients had a gross tumor or frozen section evidence of tumor, the surgical procedure was usually terminated. Exceptions to this rule were in the rare patient in whom further debulking was deemed to be possibly beneficial. Specimens from all patients were presented to a gynecology, pathology, radiation oncology tumor conference, where review of histologic material was done and staging was assigned. If all specimens were negative for neoplasm, further chemotherapy was not given. One of the 34 patients with a negative second-look was treated with radioactive chromic phosphate. Patients with persistent disease were treated with a variety of second-line modalities.

Results

Of 196 patients with epithelial ovarian carcinomas, only 65 (33.2%) had a second-look laparotomy. Progressive disease during chemotherapy was the primary reason for not performing second-look procedures (Table I). Eight of the 196 patients (4.1%) refused a second-look and are alive, all more than 36 months, without evidence of recurrent disease. Five patients died of toxicity from chemotherapy and never had a second-look operation. Ten patients were operated on who had a known tumor, as further tumor reductive surgery was contemplated. Patients with early stage (I and II) tumors of low malignant potential were not included in the study group. However, nine patients

Table V. Residual tumor from original surgery and status of second-look laparotomy

Residual	*No. of patients (%) with positive second-look*	*No. of patients (%) with negative second-look*
None	2 (29)	5 (71)
≤2 cm	16 (37)	27 (63)
>2 cm	13 (87)	2 (13)
Totals	31	34

Table VI. Chemotherapy and status of second-look laparotomy

Agent	*No. of patients (%) with positive second-look*	*No. of patients (%) with negative second-look*
Melphalan	1 (16)	16 (84)
Chlorambucil	—	1 (100)
CAP	16 (59)	11 (41)
MECY	6 (100)	—
Cisplatin, other combinations	2 (29)	5 (71)
CTX, 5-FU, Hmm	4 (80)	1 (20)
Totals	31	34

CAP = Cyclophosphamide, Adriamycin, cisplatin; MECY = High dose methotrexate with rescue plus cyclophosphamide; CTX, 5-FU, Hmm = cyclophosphamide, 5-fluorouracil, hexamethylmelamine.

with stage III borderline tumors did have second-look procedures and were included.

The average age of the 65 study group patients was 46.9 years. White patients constituted 67.7% of the patients; black patients, 24.6%; and Malaysians, 7.7%. Patients with original stage I, II disease constituted 36.9% of the patients, as compared with 63.1% of patients with stage III, IV (Table II). Forty-eight percent of the patients had a positive second-look operation, and 10 of these 31 (32%) had only microscopic disease. One patient had a microscopic neoplasm noted in a paraaortic node only. Of patients with gross disease, 52% had widespread intra-abdominal metastases, 33% were thought to have disease confined to the pelvis, and three patients had palpable disease in the omentum or nodes. Of these latter 10 patients, disease could have been in other areas, since the procedure was usually terminated when a neoplasm was found. The chances of having a positive second-look operation was directly related to the original stage of the disease. Of patients with original stage I, II disease, 25% had a positive second-look, contrasting with 60.9% of patients with stage III, IV (Table II).

Other factors that might influence the possibility of having a positive second-look laparotomy were analyzed. Twenty-nine patients were aged ≤40 years, and 44.8% had a positive second-look operation, which contrasted with 19 of 36 patients (52.8%) over age 40 years who had a positive second-look laparotomy. Patients with grade 3 lesions constituted 61.3% of all patients with a positive second-look procedure. If a patient had a grade 3 carcinoma, she had a 63% chance of having a positive second-look procedure, compared with 20% for grade 1 (Table III). As expected, the vast majority of patients had a serous cystadenocarcinoma, and patients with serous tumors constituted 64.5% of all patients with a positive second-look procedure. If one had a serous tumor, chances of having a positive second-look operation were about 50%, which contrasted with 14% for mucinous tumors (Table IV). The amount of disease left behind at the original operation was clearly related to the positivity of the second-look operation. Eighty-seven percent of patients who had tumor nodules >2 cm left behind had a positive second-look procedure (Table V).

The majority of patients who had a negative second-look procedure were treated with a single alkylating agent, and most of these patients had early stage, early grade neoplasms (Table VI). The number of courses of treatment given did not influence the positivity or negativity of the procedure; 91% of the patients received from 8 to 12 courses of therapy. At the time of this analysis, all but six of 31 patients with a positive second-look procedure were dead from disease. Seventy-four percent were dead within 3 years of the original diagnosis. One patient died 42 months after the original diagnosis; one died 6 years afterward. Of the other six patients, one has been lost to follow-up and presumed dead. Two patients are without evidence of disease at 26 and 30 months after the institution of second-line chemotherapy. Both patients had grade 1 tumors with only microscopic disease found at the second-look laparotomy. Three patients have had positive third-look procedures and are alive but have disease, and all have tumors of low malignant potential, which were originally stage III.

All patients with a negative second-look laparotomy have had follow-up at least 30 months after the procedure. Eighty-two percent have had follow-up for more than 3 years. Of patients with negative second-look operations, 24% have had recurrent disease from 5 to 23 months after the procedure. These recurrences were in liver parenchyma or in distant sites in 63% of the patients (Table VII). All of these patients are dead from recurrent disease. Additionally, one patient with a negative second-look operation died of sepsis as a result of a chemotherapy-related myeloproliferative

Table VII. Characteristics of patients with recurrent cancer after negative second-look procedures

Patient	*Age*	*Original stage*	*Residual tumor after primary operation*	*Original grade*	*Histology of tumor*	*Interval to recurrence (mo)*	*Site of recurrence*
1	46	IV	≤2	3	Serous	7	Abdominal cavity
2	65	III	≤2	3	Adenocarcinoma, poorly differentiated	5	Lungs
3	56	III	≤2	3	Serous	14	Abdominal cavity, lesser sac
4	60	IIc	None	2	Serous	6	Abdominal cavity, liver parenchyma
5	49	III	≤2	2	Serous	8	Abdominal cavity
6	71	III	≤2	3	Serous	12	Cerebellum
7	43	III	≤2	3	Serous	18	Lungs, liver, parenchyma
8	62	III	≤2	3	Serous	23	Cuff, liver, parenchyma

disorder. She had no clinical evidence of recurrent tumor.

Although no operative deaths were noted, significant perioperative morbidity occurred (Table VIII). Bowel resections after the second-look operation were required because of adhesions that caused small bowel obstruction. One patient had all but 48 inches (122 cm) of small bowel removed because of dense adhesions and tumor involvement. She had a normal preoperative computed tomography scan. The patient with vascular collapse had received only 400 mg/m^2 of Adriamycin. The average number of days spent in the hospital by patients undergoing second-look laparotomy was 8.3 with a range of 6 to 20.

Table VIII. Complications of second-look laparotomy

Complication	*No. of patients*	*Percent*
Ileus*	10	15.4
Small bowel resection required because of adhesions	9	13.8
Bowel resection required after second-look	2	3.1
EBL requiring transfusion†	10	15.4
Pulmonary (atelectasis)	2	3.1
Urinary tract infection	2	3.1
Wound infection	2	3.1
Intraoperative vascular collapse	1	1.6

EBL = Estimated blood loss.
*Ileus that requires nasogastric suction for >72 hours.
†Three of these patients lost >1000 ml of blood.

Comment

In this series, only those patients with second-look procedures for epithelial ovarian carcinoma were analyzed, and patients operated with a known tumor and the surgeon's intent of cytoreductive surgery were not included. Morbidity was significant, and a positive second-look procedure was noted in 48% of all patients. The great majority of patients with macroscopic disease at second-look procedures ultimately die from disease. "Routine" second-look procedures may not be indicated for all patients. There may be a select group of patients who are not candidates for second-look laparotomy. Is it possible to identify this group of patients?

Berek et al.[5] noted that patients aged <50 years tended to have negative second-look procedures. We found no such trend in this series of patients when those aged ≤40 years were compared with the older group. Positive second-assessment procedures were noted in 59% of patients adjuvantly treated with a combination of cyclophosphamide, Adriamycin, and cisplatin, as compared with 15% of patients treated with a single alkylating agent. The histologic type of tumor did not appear to affect the positivity of the second-look procedure, except for the small number of patients with mucinous tumors. Curry et al.[6] noted that patients with serous tumors had a 43% chance of having a positive second-look procedure, as did 54% of the patients reported by Roberts et al.[9] In the latter series, none of the seven patients with mucinous tumors had a positive second-look procedure.

Suboptimal resection of the tumor at the original operation was directly related to chances of having a positive second-look procedure, and this has been noted in almost every series of patients analyzed since 1980.[4, 5, 8-10, 12] Eighty-seven percent of the patients with residual tumors >2 cm reported here had a positive secondary surgical assessment. Several investigators have noted, as we did, that as the grade of the tumor increased, the chances of having a negative second-look procedure decreased.[4, 5, 8] In adding our patients to seven series of patients reported since 1980 with stage III, IV disease, the second-look procedure was positive in 70% of 371 patients (Table IX). The profile

Table IX. Outcome of second-look laparotomy in stage III, IV ovarian carcinoma

Author	Total No. of patients	No. with positive SLO	Percent
Schwartz and Smith[3]	128	102	80
Curry et al.[6]	14	8	57
Webb et al.[4]	29	19	66
Roberts et al.[9]	27	19	70
Ballon et al.[11]	21	12	57
Berek et al.[14]	56	38	68
Barnhill et al.[8]	55	37	67
Present series	41	25	61
Totals	371	260	70

SLO = Second-look operation.

of a patient with a significant chance of having a positive second-look procedure includes anyone with a grade 3 lesion, with stage III neoplasm, and/or with suboptimal tumor resection at the original procedure. Every effort should be made to use noninvasive techniques to rule out persistent, recurrent disease in these high risk patients.

Debulking at the time of a second-look procedure in asymptomatic ovarian cancer patients with a known tumor is somewhat controversial. In a small number of patients, Vogl et al.[13] found that surgical resection of bulky ovarian cancer at second-effort surgery did not result in long-term survival of patients. Berek et al.[14] identified a group of patients who would not benefit from secondary cytoreductive surgery: those with >1.5 cm of tumor left behind after the original procedure; those with >1000 ml of ascites or those with tumor size noted at the second-effort procedure to be >5 cm; and those with clinical evidence of recurrence. Copeland et al.[15] noted prolonged progression-free intervals and a 5-year survival of 85% in patients with only microscopic disease found at second-look laparotomy. No comment can be made regarding the efficacy of tumor reductive surgery at second-effort procedures in the patients reported in this article.

CT scan, along with fine-needle aspiration, was helpful in identifying a tumor, precluding a second-look operation in only five of our patients (Table I). In the absence of specific symptoms, a barium enema or an intravenous pyelogram was not helpful in identifying nonpalpable tumors. A CT scan with contrast should provide as much information as isolated contrast studies. CT may be helpful in identifying parenchymal liver lesions, omental involvement, or ascites that is not clinically evident.[16] CT is incapable of accurately detecting tumor nodules <1 cm, and the false-negative rate is extremely high. Anatomic distortion from prior surgery may yield false positive results; positive CT findings should be confirmed by fine-needle aspiration. A normal CT cannot replace a second-look procedure for accurate assessment of the status of the disease. After administering anesthetic to the patient, careful pelvic examination with transvaginal fine-needle aspiration before laparotomy may be of help. Cul-de-sac aspiration cytology has been shown to have as high as a 92% correlation when compared with second-look laparotomy.[17] This technique is well-tolerated by patients and is certainly of value if aspiration cytology is positive. A sensitive serologic tumor marker for evaluation of disease status and determination of the presence of persistent carcinoma would be an ideal monitoring tool. Niloff et al.[18] noted that a calcium 125 level >35 U/ml was a strong predictor of the presence of disease. Patients with high levels of calcium 125 could be spared a second-look operation. A positive laparoscopy can avoid more extensive operations in about one third of patients with ovarian cancer.[19] However, a negative or inadequate procedure is not sufficient to rule out persistent disease, as there are obviously limitations of observations of the anterior abdominal wall and retroperitoneal lymph nodes.

No one in this series had a second assessment procedure after irradiation, but major complications were noted in a significant number of patients. Febrile morbidity, wound infections, and ileus are the complications most frequently noted, but the details of complications are not given in some reports. One report from England noted that 70% of 50 patients had ileus, 54% required transfusions, and one had a bowel resection.[7] A report from Schwartz and Smith[3] indicated 9% had wound infections, 5% had bowel injuries or obstruction, and 8% had significant hemorrhage. Roberts et al.[9] noted a 4% incidence of ileus, a 7% incidence of wound infections, and 4% who required transfusion. One patient had a bowel obstruction. These complications reported here and by others underscore the need for further development of noninvasive methods to detect subclinical recurrences.

Of the 34 patients with a negative second-look laparotomy, 24% have had a recurrence. This figure is not much different from other reports, ranging from a low recurrence rate reported by Webb et al.[4] and Barnhill et al.[8] of 12.5% to a high of 22.2% reported by Berek et al.[5] All of our patients had recurrences within 23 months of the second-assessment operation. Most patients with a recurrence will manifest disease within 2 years of the second-look operation,[5,6] although Barnhill et al.[8] reported recurrences in two of six patients after a period of 3 years. Two of their six patients with recurrence had endometrioid tumors, whereas all but one of our patients with recurrence had serous tumors. Roberts et al.[9] noted that residual disease after original tumor-reductive surgery was a very important factor

in patients who developed a recurrence after a negative second-look procedure. If residual disease was present, 60% recurred, as opposed to 7% who had no residual disease. Only one of our eight patients with recurrence had no gross residual disease after the original procedure. The second-look laparotomy should be thorough and should include pelvic and para-aortic lymph node sampling. Berek et al.[5] have reported patients with isolated microscopic disease in retroperitoneal nodes without evidence of intraperitoneal disease at the time of second-look laparotomy. All of the patients with negative second-look operations reported here, who later had recurrences, had at least pelvic node sampling. Certain factors are associated with an increased risk of recurrence: (1) stage III disease or more, (2) any residual disease after the original tumor-reductive surgery, (3) advanced grade of tumor, and (4) serous tumors.

Sixty-three percent of our patients with a negative second-look procedure had a later recurrence in the liver or distant sites. Others[5, 8] have also reported recurrences in the liver and inguinal nodes. Regular follow-up at close intervals is indicated in patients with negative second-look procedures and should include chest x-ray films, serum tumor markers when available, liver function studies, and CT scans, in addition to careful pelvic examination.

In summary, patients with early stage, low grade lesions are at low risk for having a positive second-look procedure, as are patients with early stage mucinous cystadenocarcinoma. Patients with grade 3 lesions, stage III lesions, and/or suboptimal resection of the original tumor should undergo every available screening test before submitting themselves to a second-look procedure, since more than two-thirds will have a positive second-look result. Survival of patients with a gross tumor at a second-look operation appears to be dismal, no matter what second-line treatment is given. Second-look celiotomy is not a procedure without major complications and should be performed in tertiary care centers. A group of patients can be identified who are at greatest risk to have a recurrence after a negative second-look procedure and includes those with advanced stage, high grade serous tumors who have any residual tumor left behind at the original procedure.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. KENNETH MUHLENDORF, Norfolk, Virginia (Official Guest). Dr. Gallop and his colleagues have shared with us a multi-center retrospective review of a large number of patients undergoing second-look procedures for ovarian epithelial cancer over a 10-year period. In addition to the usual reporting of patient outcome, Dr. Gallop also considers the significant morbidity of these operations and the unfortunate 24% of patients who have a relapse within 2 years after a negative second-look procedure.

There are several lessons to be learned from this study that apply directly to gynecologists who provide primary care. This article stresses the necessity for a

meticulous investigation before a second-look operation is done. It is indeed frustrating that so many patients who undergo these operations have lethal amounts of disease remaining for which so little can be done. I was fascinated by the suggestion of using cul-de-sac aspiration cytology to help detect microscopic disease before surgery. Do you think the day will come when cul-de-sac aspirated cells can be grown in tissue culture, much as amniotic fluid cells are grown, and then used to determine the best treatment regimen, much as we do with urine culture and sensitivities?

As others have shown, the response to initial chemotherapy and the probability of a negative second-look operation increased significantly if the first surgery removed all of the disease. In this study, where there was no residual disease, 71% of the patients had a negative second-look procedure. Unfortunately, ovarian epithelial malignancy cannot be diagnosed accurately with preoperative studies. Often enough, a surgeon finds himself confronted with complex intra-abdominal disease that requires skill outside of the experience of many pelvic surgeons in practice. To obtain the results presented in this article, a high index of suspicion and appropriate referral to tertiary care physicians are essential.

Patients in this study who are treated with a single alkylating agent were more likely to have a negative second-look procedure than those treated with combination chemotherapy. Do you find that the overall long-term survival remains better with single agent therapy?

The great weakness of the second-look procedure is the relatively few patients who actually benefit. Because the intended goal is to find the patient for whom all treatment may stop, the high morbidity, not to mention the cost of this surgery, pleads for some magic bullet. Calcium 125 is not sensitive or specific enough. Modern CT scans provide resolution to 1 cm but cannot differentiate the enlarged lymph node from one that contains a tumor. It appears that magnetic resonance imaging cannot provide a signal intensity sufficient to detect subclinical ovarian cancer. Just as the CT scan is proving in many cases to have more value than the intravenous pyelogram, perhaps soon the use of antibodies with ovarian tumor antigens can replace the second-look procedure.

Until the perfect tumor marker is discovered or created in the laboratory, the gold standard for determining the cure of ovarian cancer remains the meticulously performed second-look operation done by a skilled surgeon with a skilled pathologist. With this must be added the inevitable variable of time, for it is the longevity of the disease-free interval that ultimately allows one to declare a patient cured.

I congratulate Dr. Gallop for a thorough review of an accepted technique with attention to its limitations and its complications.

Dr. Paul B. Underwood, Charlottesville, Virginia. I would like to ask you two questions. (1) Was there a difference in the recurrence rate in the patients treated with single agent Alkeran as compared with your count? (2) On the negative second-look operations, do you treat those patients with any further methods?

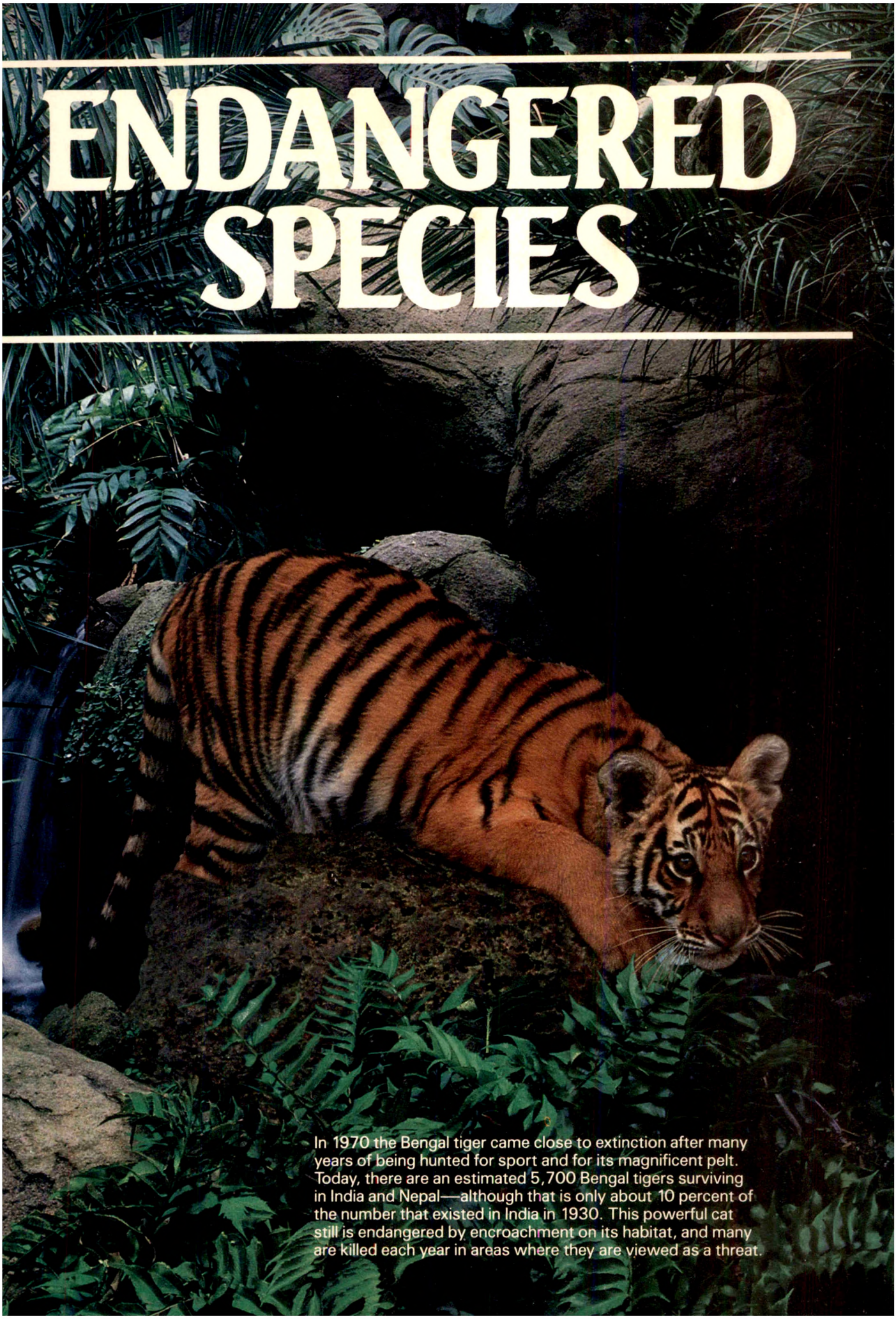
Dr. Matthew Burrell, Atlanta, Georgia. Did you find any higher risk for the clear cell adenocarcinoma? Our group has found that clear cells are a very high risk predictor of residual findings on second-look procedures.

Dr. William T. Mixson, Coral Gables, Florida. Questions are being asked by the oncologist. As a generalist I am sitting here thinking that this is almost an article that says that a second-look operation may not be very good. How many of these people are helped? How many benefit from the use of the second-look operation?

Dr. Gallup (Closing). The value of cul-de-sac aspiration cytology was recognized by Dr. Keetle many years ago. In fact, drug sensitivity is in its infancy. It may help us in the future. Other reports, besides ours, indicate significant long-term survival in patients treated with single alkylating agents. In general, these patients all have early grade, early stage lesions. Recent work in this country leans toward recommendations for multi-agent drug therapy for the majority of patients with advanced epithelial ovarian tumors. We may not be really improving the walking, talking 5-year survival, but we are improving the progression-free interval. Ten years ago, a patient may have died within 2 years of diagnosis. Now, a patient with a similar lesion may live a few years longer with fairly good quality of life. Dr. Burrell, we had no patients with clear cell tumors, so I cannot comment.

Dr. Underwood asked about treatment options for the patients with a negative second-look operation. Dr. Dudzinski, who relieved me at Portsmouth Naval Hospital, did treat one patient in this series with radioactive intraperitoneal chromic phosphate. At the Medical College of Georgia, we are selecting a group of high risk patients who probably should be treated. Maybe we should give them "three for the road," as we have done with gestational trophoblastic disease. Other options include intraperitoneal immunotherapy or chemotherapy. Remember, about one quarter of patients with appropriate negative second-look operations will have a recurrence. Whichever methods are chosen, patients should be carefully randomized. Dr. Mixson, I believe we must be very careful in selecting patients on whom we operate. If you believe it is a bulk tumor, why operate? On the other hand, patients with intestinal obstruction may benefit in select situations. Another procedure to relieve symptoms may be helpful in a few patients.

ENDANGERED SPECIES



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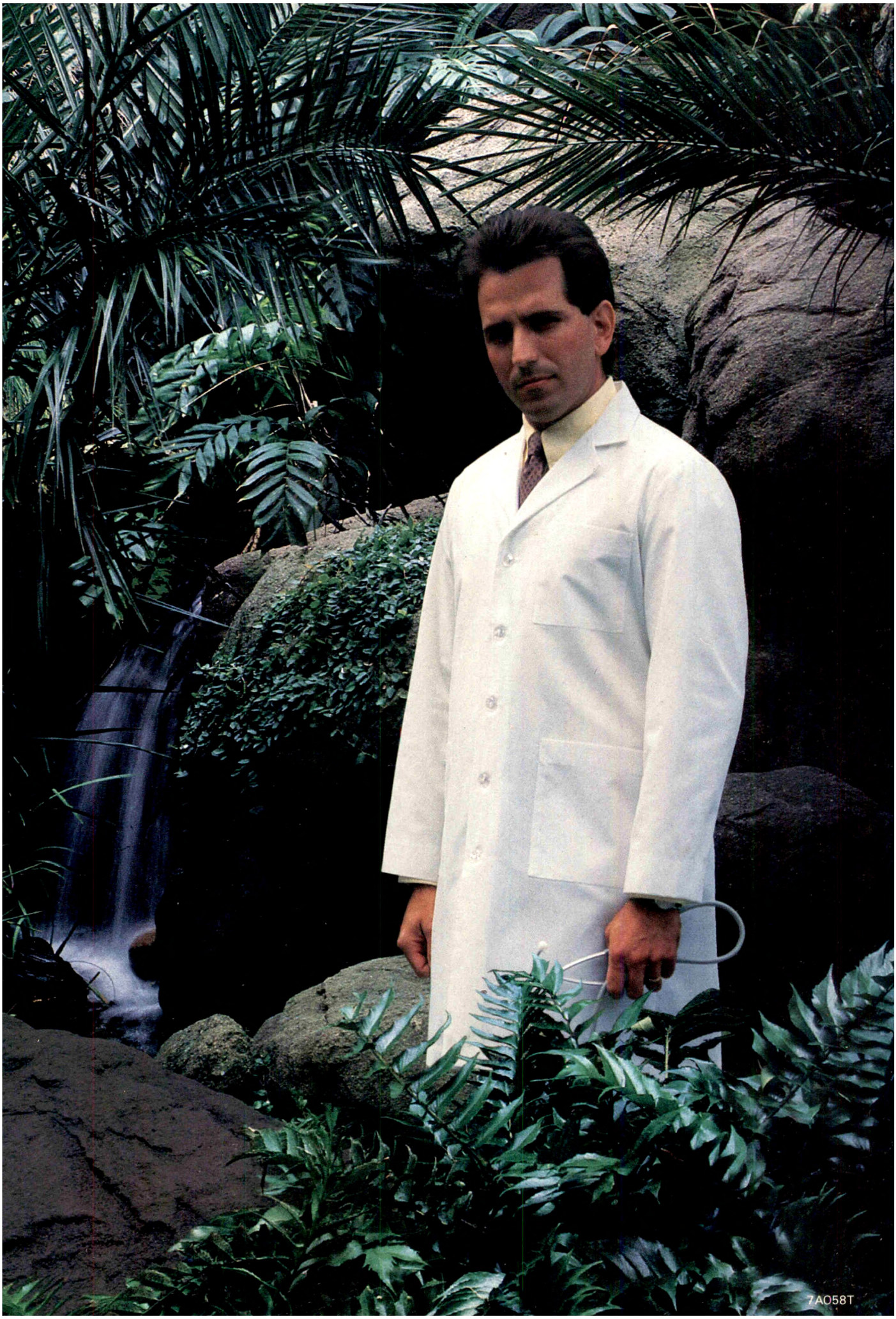
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Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers	10.2	2.6		62.0	15.9
≥15 cigarettes daily	13.0	5.1		78.7	31.3
<15 cigarettes daily	4.7	0.9		28.6	5.7
Nonsmokers	1.8	1.2		10.7	7.4
Smokers and nonsmokers	5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low. Age:	<30	30-39	≥40
Heavy smokers (≥15/day)	C	B	A
Light smokers (<15/day)	D	C	B
Nonsmokers, no predispositions	D	C,D	C
Nonsmokers, with predispositions	C	C,B	B,A

Be alert to earliest manifestations of TE and thrombotic disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. The RCGP prospective study reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. The data suggest varicose veins have little effect on development of deep vein thrombosis but substantially increase risk of superficial vein thrombosis, risk depending on severity of varicosities. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is evidence that estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions (eg, irregular bleeding before OCs, polycystic ovaries) occurr nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer w combination or progestogen-only OCs, though individual cases were reported. Studies show no increased breast cancer with OCs or estroge However, greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk of bre cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens rose and further increas with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other risk factors. Decrease in benign bre tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, erosion, and carcinoma in long-term use Cervical microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increased canc risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consic nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of breast canc or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. **4.** *Hepatic adenomas, e* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk, as was age ov 30. Though benign, these lesions may rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk duration of use, being much greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,00 Consider such lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to half wi acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT m be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. OCs are contraindicated in case of past present, benign or malignant liver tumors developing during use of estrogenic products. **5.** *Use in or immediately preceding pregnancy, bir defects, malignancy in offspring:* Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in ute to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monit such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DE exposed daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Other estroger may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL for vertebral, anal, cardia tracheoesophageal, renal, and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of limi reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threatene abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, includin OCs, during early pregnancy occurred in 19.2/1,000 births, compared to 7.8 for those unexposed ($P<.05$). More twinning was reported conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but there is considerabl evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased abortuses from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion increased soon after stopping OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not bee demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods ar missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. pregnancy is confirmed, apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy shoul use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not us progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 years use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. **7.** *Carbohydrate and lipid metabolism:* Decreasec glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides and tota phospholipids may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few months, and degree may relate to progestoger dose. Hypertension prevalence in users is not higher in the first year, but increases with longer exposure; in the 5th year it is 2½—3 time the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), preexisting rena disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weigh gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Eve though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors fo cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of migraine o development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating cause. **10.** *Bleedin irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregula bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology i excluded, time or change to another product may suffice. Change to a higher-estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. There may be increased risk of ectopics after discontinuing. **12.** *Breast feeding:* OCs may decrease quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after weaning. **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable difference in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC and troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake, unaltered free T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceride, phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask onset of climacteric. Cervical chlamydial prevalence is increased; do not assume OC protects against chlamydial PID. *Information for patients.* See patient labeling. *Drug interactions:* OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophen. **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted for: premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hemolytic uremia, malignant hypertension, itching, vaginitis, porphyria, impaired renal function, acute renal failure, sometimes irreversible, PVCs, abnormal EKG, pulmonary hypertension. TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythematosus, rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanoma, endometrial, cervical, and breast carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use and is available on request.**

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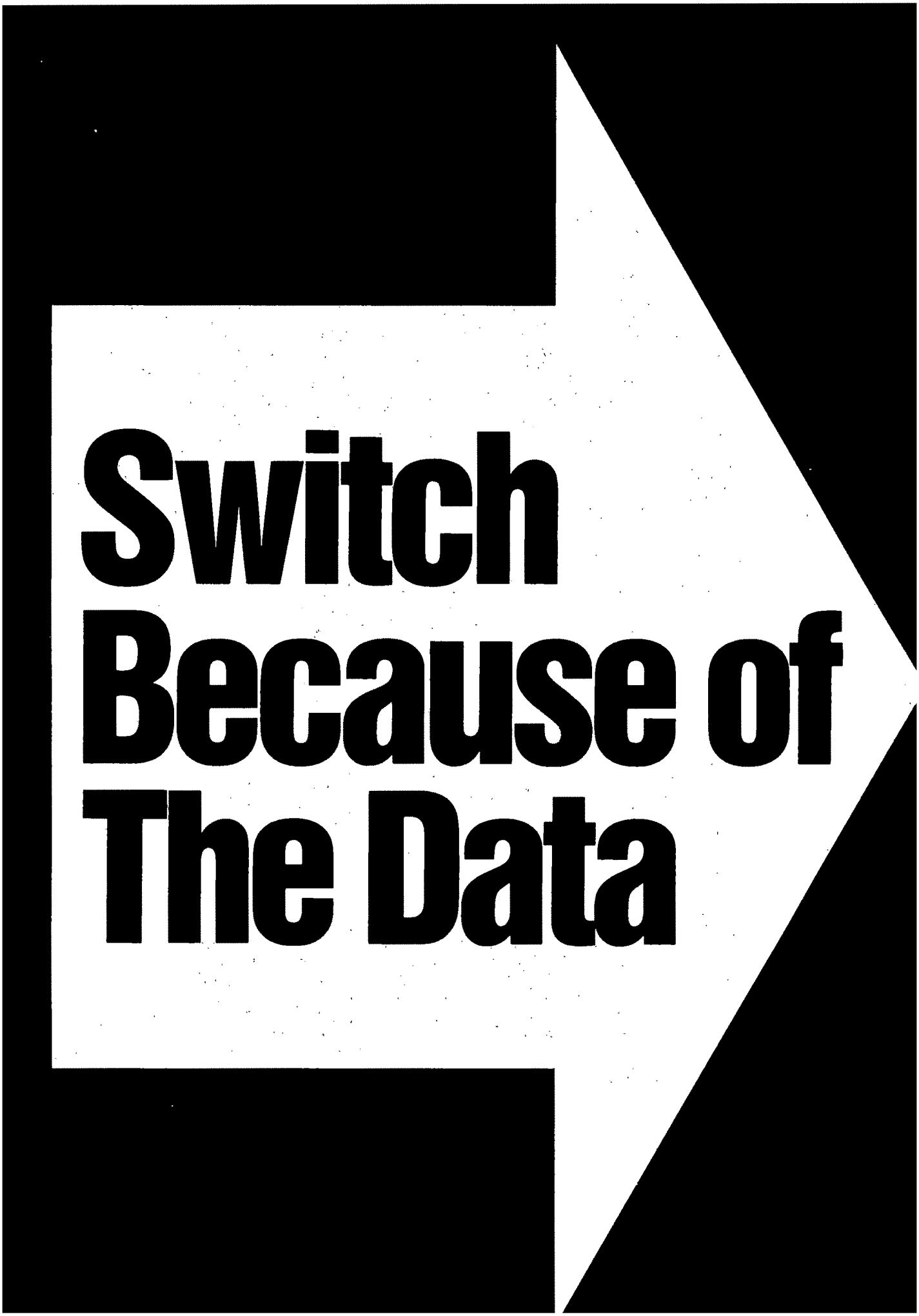
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URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Pseudomonas* sp. including *Ps. aeruginosa; Proteus mirabilis; P. vulgaris; Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp.; *Serratia* sp. including *S. marcescens; Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae.*

INTRA-ABDOMINAL INFECTIONS caused by *Escherichia coli; Staphylococcus epidermidis; Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Bacteroides* sp. including *B. fragilis; Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis; Escherichia coli; Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis; Serratia* sp.; *Enterobacter* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis; Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae.* 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae.*

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who developed diarrhea in association with antibiotic use. Treatment with broad-spectrum antibiotics alters normal flora of the colon and may permit overgrowth of *Clostridia.* Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro.* Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile.* Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Careful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox,' nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established.

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should be exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not been established. In children six months of age and older, treatment with 'Cefizox' has been associated with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatine phosphokinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the *most* frequent adverse reactions (*greater than* 1%, but less than 5%) are: *Hypersensitivity*—Rash, pruritus, fever. *Hepatic*—Transient elevation in SGOT, SGPT and alkaline phosphatase. *Hematologic*—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. *Local*—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (*less than* 1%) are: *Hypersensitivity*—Numbness has been reported rarely. *Hepatic*—Elevation of bilirubin has been reported rarely. *Renal*—Transient elevations of BUN and creatinine have been occasionally observed with ceftizoxime. *Hematologic*—Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. *Genitourinary*—Vaginitis has rarely occurred. *Gastrointestinal*—Diarrhea; nausea and vomiting have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams of ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxime.

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams of ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufactured for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

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References: 1. Lou, M.A., et al.: Comparative evaluation of ceftizoxime and cefoxitin in the treatment of intraabdominal infections, 14th Internat. Congr. Chemother., Kyoto, Japan, June 1985, pp. 2378-2379. 2. Fu, K.P., and Neu, H.C., Antibacterial activity of ceftizoxime, a ß-lactamase-stable cephalosporin, *Antimicrob. Agents Chemother.* 17:583-590, 1980. 3. Thornsberry, C.: Review of in vitro activity of third-generation cephalosporins and other newer beta-lactam antibiotics against clinically important bacteria, *Am. J. Med.* 79(Suppl. 2A): 14-20 (Aug.) 1985.

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Endometrial metaplasia associated with endometrial adenocarcinoma

Willie A. Andersen, M.D., Peyton T. Taylor, Jr., M.D., Robert E. Fechner, M.D., and Jo Ann V. Pinkerton, M.D.
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Endometrial metaplasia is a complex group of epithelial proliferations. The relationship of metaplasia, other than squamous metaplasia, to endometrial adenocarcinoma has not been clearly established. Between 1969 and 1979, 183 patients diagnosed as having Stage I endometrial adenocarcinoma (according to International Federation of Gynecology and Obstetrics) were treated at the University of Virginia Medical Center. Sixty of the patients were treated with hysterectomy without preoperative irradiation. A histopathologic review was performed without knowledge of the clinical outcome and subsequent clinopathologic correlations were analyzed. On review, 32/60 had carcinoma without metaplasia and 15/60 had both carcinoma and metaplasia. Thirteen of the 60 patients were judged not to have cancer: 12 had both hyperplasia and metaplasia and one had hyperplasia without metaplasia. None of the 12 patients reclassified as having metaplasia had a recurrence or died of endometrial carcinoma. Patients with both metaplasia and carcinoma were significantly younger than patients with only carcinoma and the associated carcinomas were more frequently well differentiated. (AM J OBSTET GYNECOL 1987;157: 597-604.)

Key words: Endometrial carcinoma, endometrial metaplasia

The human endometrium has a very wide range of histologic expressions in normal, hyperplastic, and neoplastic conditions. In spite of extensive studies spanning several decades, there is still controversy over the histologic criteria of endometrial adenocarcinoma.[1-3] In 1980 an additional complication was added by Hendrickson and Kempson[4] when they described the range of benign proliferative changes of the endometrium which they collectively called "nonneoplastic endometrial metaplasias." Although the familiar "squamous" metaplasia was included in their work, the focus was on "new" metaplastic processes, which included eosinophilic, mucinous, papillary, tubal, and clear cell types. Does the identification or segregation of these metaplastic changes have any importance or does it only add further confusion? This report reviews cases in which the diagnosis was Stage I adenocarcinoma of the endometrium with treatment by hysterectomy and discusses the relationship of metaplasia to carcinoma in these patients.

Material and methods

Between 1969 and 1979, 183 patients with Stage I adenocarcinoma of the endometrium (International Federation of Gynecology and Obstetrics, FIGO) were identified through the tumor registry of the University of Virginia Medical Center. During this time period, 123 patients with adenocarcinoma clinically confined to the uterus were treated with preoperative radiation followed by exploration and hysterectomy. For this reason, there were only 60 cases where there was a hysterectomy specimen without the confounding changes of irradiation. All available histologic material from the endometrial biopsy or curettage and the hysterectomy specimen was reviewed without knowledge of the previous diagnosis, treatment, or clinical course of the patient. The clinical records were abstracted, coded, and analyzed by use of the Statistical Package of the Social Sciences system of the Vogelbach Computing Center of Northwestern University, Version 9.0, 1984. Statistical significance was determined by χ^2 analysis of contingency tables. Statistical significance was set at the 5% probability level.

All cases were staged as FIGO Stage I. After hysterectomy, 17 patients received postoperative radiation therapy after surgical-pathologic correlation. No patients were lost to follow-up.

The criteria for diagnosis of the various forms of metaplasia were those of Hendrickson and Kempson.[4] Representative photomicrographs of metaplasia are illustrated in Figs. 1 to 4.

The designation of hyperplastic endometrium required abnormal amounts of proliferative endometrial tissue, architectural complexity, or cytologic atypia. The

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Reprint requests: Peyton T. Taylor, Jr., M.D., Box 387, University of Virginia Medical Center, Charlottesville, VA 22908.

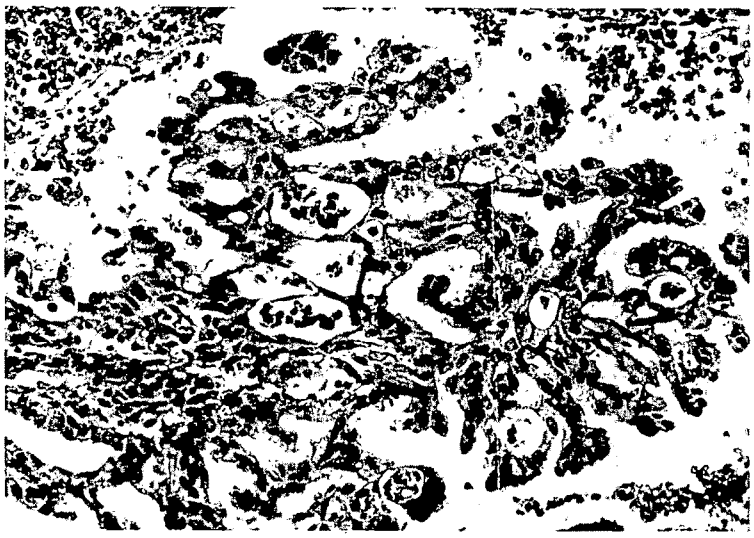


Fig. 1. Syncytial papillary metaplasia consisting of uniform benign cells lacking well-defined borders that form small sheets and well-defined papillary projections. Neutrophils are often intermixed with the epithelium. (Hematoxylin and eosin. Original magnification ×125.)



Fig. 2. Tubular (ciliated) metaplasia characterized by cells with a variegated appearance resembling epithelium of the normal salpinx. Many of these cells contain cilia *(arrow)*. Endometrial stroma surrounds most glands. (Hematoxylin and eosin. Original magnification ×125.)

classical adjectives of "cystic glandular," "adenomatous," and "atypical" were used.

The designation of adenocarcinoma required architectural and cytologic abnormalities as defined by Hendrickson and Kempson.[5]

Results

The diagnosis and interrelationship of metaplasia and cancer are given for both the biopsy/curettage specimen (Table I) and the hysterectomy specimen (Table II).

After the above criteria were applied, the diagnosis on the curretings from 13 patients (21%) was changed from carcinoma to hyperplasia without carcinoma. Eight had hyperplasia with atypia, four had adenomatous hyperplasia without atypia, and one had cystic hyperplasia without atypia. Eleven of these patients also had metaplasia. Six of the eight with hyperplasia and atypia had metaplasia as did all four of those with adenomatous hyperplasia and the one patient with cystic hyperplasia. None of these patients had carcinoma in the hysterectomy specimen and none had recurrence



Fig. 3. Eosinophilic metaplasia, which includes a cuboidal cell with eosinophilic cytoplasm and a centrally located, normal nucleus. The cells are all the same size and surrounded by endometrial stroma. (Hematoxylin and eosin. Original magnification ×125.)

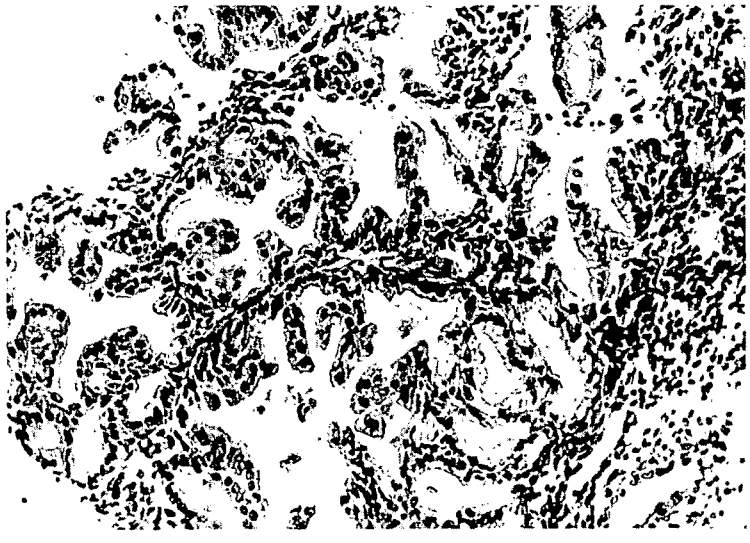


Fig. 4. Mucinous metaplasia that has small cells with basal nuclei and mucus-filled cytoplasm on the luminal side of the cell. Variable amounts of endometrial stroma are adjacent to the stroma. (Hematoxylin and eosin. Original magnification ×125.)

or died of endometrial carcinoma. The median follow-up for this group is 80 months with a range of 25 to 139 months.

Forty-seven patients had endometrial carcinoma on curettage (Table I). Thirty-two had carcinoma without metaplasia and 15 had both carcinoma and metaplasia. The most common pattern of carcinoma was endometrioid (28 patients). This was followed by "mixed" carcinomas (10 patients), serous papillary carcinoma (four patients), adenosquamous carcinoma (two patients), clear cell carcinoma (two patients), and poorly differentiated small cell malignancy (one patient). All 10 cases of "mixed" carcinoma had an endometrioid component; eight cases had a serous papillary component and two cases had a mucinous component.

The types of endometrial carcinoma associated with the presence or absence of metaplasia are shown in Table III. Although no statistically meaningful correlation could be drawn, there was always some component of "endometrioid" carcinoma associated with metaplasia.

The different patterns of metaplasia varied between

Table I. Review diagnoses of endometrial biopsy/curettage specimens (N = 60)

Carcinoma on curettings	*Metaplasia on curettings*		
	Yes	*No*	*Total*
Yes	15	32	47
No	11	2	13
Total	26	34	60

Table II. Review diagnoses of hysterectomy specimens (N = 60)

Carcinoma in uterus	*Metaplasia in uterus*		
	Yes	*No*	*Total*
Yes	15	25	40
No	12	8	20
Total	27	33	60

the curettage and the hysterectomy specimen (Tables IV and V), probably reflecting a greater amount of histologic material for study in the latter. Although metaplasia was commonly seen as a single type, multiple types were seen in 52% of the curettage samples and 42% of the hysterectomy samples. There was no single type or combination of types of metaplasia more likely to be associated with malignancy. Both eosinophilic and ciliated metaplasia were found more frequently than squamous metaplasia.

When metaplasia and malignancy coexisted in a curettage specimen, the malignancy was more likely to be well differentiated (85% grade 1) than in those cases without metaplasia (68% grade 2 or above). These differences did not achieve statistical significance ($p = 0.06$). The presence or absence of metaplasia did not correlate with the depth of invasion of the carcinoma into the myometrium. Although a "pushing" margin was seen more often when cancer and metaplasia coexisted and an "infiltrative" pattern was more typical of cancer alone, the correlation was not of statistical significance.

Patients with only metaplasia were significantly younger (57 ± 2.1 years) than patients with metaplasia and cancer (60 ± 1.3 years) or patients with cancer only (65 ± 2.1 years) ($p = 0.04$).

Table VI shows the associated nonneoplastic diagnoses when only metaplasia was seen in the endometrial curretings. The finding of some "normal" endometrium was significantly correlated with the absence of carcinoma ($p = 0.01$).

Neither a lymphocytic stromal infiltrate nor stromal foam cells correlated with the finding of carcinoma in the curettings. A desmoplastic response in the curettings was likely to be associated with cancer and was noted in only one of the 11 cases of "pure" metaplasia. The correlation of desmoplastic reaction and cancer did not, however, achieve statistical significance ($p = 0.06$).

Table III. The relationship of metaplasia and histologic type of carcinoma diagnosed in endometrial biopsy/curettage specimens

Histologic type	*Cancer without metaplasia*	*Cancer with metaplasia*	*Total*
Endometrioid	17	11	28
Adenosquamous	2	0	2
Clear cell	2	0	2
Papillary serous	4	0	4
"True mixed"	6	4	10
Other	1	0	1
Total	32	15	47

There were nine deaths caused by cancer in the study group of 60 women. Only two of these occurred in patients with both metaplasia and carcinoma. None of the patients with only metaplasia in the curettings had carcinoma of the uterus, and none of these women had a recurrence or died of endometrial carcinoma. The life-table survival for all women with cancer (with or without metaplasia) was 81% ± 5.8%. The corresponding figure for those with cancer and metaplasia was 91% ± 6.2% while those with cancer and no metaplasia had a 70% ± 10.8% survival prediction. These differences do not achieve statistical significance ($p = 0.09$).

Comment

The endometrial metaplasias were thoroughly described by Hendrickson and Kempson[4] in 1980 but there have been only a few subsequent discussions.[6-8] The cases reported by them were, however, specifically selected to exclude carcinoma. We believe this has led some to conclude that the diagnosis of metaplasia precludes the possibility of a coexisting carcinoma of the endometrium, especially a carcinoma which could invade the myometrium.[1,9] The present series emphasizes that metaplasias coexist in endometria with either endometrial hyperplasia or carcinoma.

Our experience with FIGO Stage I carcinoma indicates that metaplasia often coexists with carcinoma: 32% (15/47) in uterine curettings and 38% (15/40) in the hysterectomy material. Metaplasia was seen almost as often as hyperplasia with cancer. Although the association of squamous metaplasia and well-differentiated adenocarcinoma has been described for decades (for example, adenoacanthoma), the coexis-

Table IV. Types of metaplasia encountered in endometrial biopsy/curettage specimens*

Metaplasia	*Patients with metaplasia and cancer (N = 15)*	*Patients with metaplasia only (N = 11)*	*Total (N = 26)*
Squamous	5	5	10
Mucinous	4	3	7
Papillary	2	2	4
Eosinophilic	11	8	19
Tubal (ciliated)	1	4	5
Clear cell	1	0	1

*Patients could have more than one type of metaplasia.

Table V. Types of metaplasia encountered in hysterectomy specimens*

Metaplasia	*Patients with metaplasia and cancer (N = 15)*	*Patients with metaplasia only (N = 12)*	*Total (N = 27)*
Squamous	3	5	8
Mucinous	1	1	2
Papillary	0	2	2
Eosinophilic	7	8	15
Tubal (ciliated)	7	10	17
Clear cell	1	0	1

*Patients could have more than one type of metaplasia.

Table VI. Nonneoplastic diagnoses associated with metaplasia on curettage*

Diagnosis	*Metaplasia with cancer (N = 15)*	*Metaplasia only (N = 11)*	*Total (N = 26)*
Cystic hyperplasia	1	1	2
Adenomatous hyperplasia	4	4	8
Atypical adenomatous hyperplasia	6	2	8
Proliferative endometrium	4	2	6
Atrophic endometrium	1	1	2
Proliferative and atrophic endometrium	1	2	3

*Patients could have more than one diagnosis.

tence of a variety of non-squamous metaplasias and adenocarcinoma has not heretofore been stressed. In our series the most common metaplasia coexisting with cancer was eosinophilic, not squamous, metaplasia.

Silverberg[1] views endometrial metaplasia as having only "dubious distinction," important only in that they not be mistaken for carcinoma. We believe that the separation of benign metaplastic changes from malignancy continues to be a critical problem. Thirteen of 60 patients (22%) who were originally diagnosed and treated as having cancer were felt to have only metaplasia and hyperplasia by current criteria. Including such patients in a therapeutic trial could lead to erroneous conclusions regarding the therapeutic outcomes.

Distinguishing metaplasias from carcinoma admittedly can be difficult. Pattern diagnosis alone is unreliable. Careful attention to the cytologic detail and awareness of the architecture of metaplasias permit their recognition. Other histologic variables were investigated for their utility in differentiating metaplasia from carcinoma, but only the presence of normal endometrium admixed with metaplasia reached statistical significance. Observed trends, however, suggested that the presence of a demoplastic response and scant or absent normal endometrium in the curettings often suggested the coexistence of cancer with metaplasia.

The clinical setting of metaplasia, with or without associated carcinoma, closely parallels that of endometrial hyperplasia. Metaplasia and hyperplasia tend to occur in relatively younger women with abnormal uterine bleeding. Chronic oligoovulation, exogenous estrogen usage, exogenous obesity, and intrauterine contraceptive device usage have all been reported to be associated with metaplasia,[4,6-8] but our data were not coded to correlate these features.

In this series, when metaplasia or hyperplasia was

associated with carcinoma, the malignancies were mostly well differentiated and were more often of the endometrioid variety. There were no instances in either the curettage material or in the hysterectomy specimens where metaplasia seemed to merge into or to be transformed into carcinoma, in spite of careful histologic review.

In our study, we had no case in which cancer was absent in the curettings but present in the hysterectomy specimen. Since completion of this review, however, we have had two patients in whom there was only nonsquamous metaplasia in the formal curettings but in whom metaplasia and carcinoma were found in the removed uterus. In both patients the carcinoma was well differentiated but invaded the myometrium, in one case more than 50% of the wall thickness.

In conclusion, metaplastic proliferations are important not only because of the need to be differentiated from adenocarcinoma but because they can be found in association with carcinoma. This association is far more rich and varied than just the familiar adenoacanthoma and both the pathologist and the clinician must be aware of the potential coexistence. If metaplasia is diagnosed in the setting of perimenopausal or postmenopausal bleeding by office endometrial biopsy or curettage, a more complete sampling (formal curettage) should be considered. Because of the coexistence of metaplasia and adenocarcinoma found in this retrospective review, we feel that the endometrial metaplasias should be viewed with concern.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. MICHAEL P. BORN, Miami, Florida (Official Guest). Endometrial metaplasia has been defined as the replacement of the endometrial glandular epithelium by an epithelium that is normally found in another müllerian-derived organ such as the fallopian tube.[1] Dr. Taylor and his colleagues asked the question, "Do the identification and segregation of these metaplastic changes have any real importance or do they only add further confusion?" The question is answered in the article's conclusion where it is asserted that the metaplasias are important because they may be confused with carcinomas and because they frequently coexist with carcinomas. Others have acknowledged these points separately[2-4] but have not highlighted their combined significance as have Dr. Taylor and his coauthors. If these conclusions are warranted, they provide ample justification for clinicians and pathologists alike to be aware of the existence and significance of the endometrial metaplasias.

The data for this paper were generated by the review of curettage and hysterectomy specimens for 60 patients treated for FIGO Stage I endometrial carcinoma at the University of Virginia Medical Center between 1969 and 1979. This review identified 13 patients who did not have carcinoma. Eleven of these 13 patients had metaplasia that presumably led to the misdiagnosis. In addition, a high frequency of coexistence of metaplasia and carcinoma was identified. The incidence of coexistence was 32% in the curettage specimens and 37.5% in the hysterectomy specimens. Certainly these data should disabuse us of any misconception that the presence of metaplasia precludes the presence of carcinoma.

The authors made additional important observations. They noted that metaplasias and hyperplasias are found in similar clinical settings, particularly that of the younger patient with abnormal uterine bleeding. Although not documented in this study, other investigators believe that metaplasias are stimulated by unopposed estrogen, both endogenous and exogenous.[3,5] Another observation of clinical interest is that the endometrial carcinomas found with coexistent metaplasia are usually well differentiated and usually of the endometrioid cell type; these patients generally have an improved 5-year survival rate when compared with that for carcinomas without metaplasia. This study was obviously retrospective. I would like to ask Dr. Taylor if he is collecting any prospective data and, if so, whether estrogen and progesterone receptors are being determined.

The 13 cases that were initially misdiagnosed, or perhaps overdiagnosed, represent 21% of the study group. This would seem a startling statistic until we recognize it as merely illustrative of the continuing difficulty pathologists have in agreeing on a classification for the proliferative endometrial lesions. In a review of 100 consecutive cases of endometrial hyperplasia referred to the Sloane Hospital for Women, 69% of the diagnoses were downgraded by the reviewers.[4] Debate con-

tinues about the existence and significance of endometrial carcinoma in situ as well as the diagnostic criteria for well-differentiated endometrial carcinoma.[6-9] In this environment of academic uncertainty, clinicians must communicate freely with their pathologists to be sure that the biologic potential of the endometrium is understood and that subsequent management is appropriate.

Hendrickson and Kempson[3] made an important distinction between metaplasias that are found in otherwise unremarkable, nonhyperplastic endometrium and metaplasias found in the midst of atypical hyperplasias, which they called "metaplastic hyperplasias." The first group should pose no problem of interpretation for the pathologist and should be considered neither malignant nor premalignant. On the other hand, the "metaplastic hyperplasias" may well present a very complex histologic picture subject to overdiagnosis. These authors believe that these "metaplastic hyperplasias" represent a subset of atypical adenomatous hyperplasias and share the generally accepted increased risk of subsequent development of adenocarcinoma. Perhaps the pivotal point is not the presence of metaplasias but rather the "company they keep."

The two cases that occurred after the review was completed raise some interesting issues. These patients are reported to have "only metaplasia" in the curettings but metaplasia and carcinoma in the hysterectomy specimens. Of greater concern is that in both cases the myometrium was invaded. I would like to pose several questions regarding these cases. If only metaplasia was diagnosed on the curettings, what were the indications for hysterectomy? Was there atypical hyperplasia in the curettings as well? Is there any theory as to why the carcinomas were missed at curettage? Finally, how can we clinicians avoid similar pitfalls?

The authors' conclusions are that the metaplasias are important because they may be misdiagnosed as adenocarcinoma and because they may be frequently associated with carcinoma. In closing, I would like to ask Dr. Taylor two final questions. First, does he accept the concept of the "metaplastic hyperplasias" and their categorization as a subset of the atypical hyperplasias, imbued with their asssociated implications? Second, would he agree that, although a potential source of confusion, the true significance of the metaplasias does not lie in their presence alone but rather is dictated by the background endometrium, whether it be atrophic, proliferative, hyperplastic, or malignant?

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DISCUSSER NO. 2. I have a great deal of respect for the quality of pathologic diagnosis at the University of Virginia and so I question whether these interpretations would occur with that quality. This is more likely to occur in our community hospitals. One of the concerns that oncologists have had is that there is a lack of respect on the part of many of us for the lethality of endometrial carcinoma. It is considered a malignancy that is readily curable. From your data, then, looking back from 1980, would it be fair to say that we should deduct from the cure rate of endometrial carcinoma some 15% to 20% that would indicate that it is indeed a more lethal disease than we have regarded it?

DR. TAYLOR (Closing). Misinterpretation of pathologic results is a serious problem. It is particularly a problem from the point of clinical trials. A large number of patients do not have very aggressive lesions however these proliferative changes are described, so for prospective trials it is very difficult to interpret the outcome. That does automatically shift survival downward. If the series is weighted with 20% very favorable lesions, the survival rate for endometrial cancer is lowered and the results do not look good. Do I accept metaplastic hyperplasias as a concept and are they judged by the "company they keep"? I think all 13 of these patients had hyperplasia. Eleven of those had metaplasia and hyperplasia. I am sorry that I did not make it clear that this is part of the "company they keep." I think what makes it so confusing is the architectural complexity of hyperplasia superimposed on metaplasia. These are very complex lesions. The examples of metaplasia with chronic infections and with intrauterine contraceptive device usage indicate that they are just a histologic response. So I would say yes, my interpretation is that they are judged by the "company they keep." Do I accept it as a concept? I think it is real, that hyperplasias do exist with metaplastic changes, but this concept is different from the hyperplasias seen with chronic infections and in younger people. As far as the two patients that I mentioned after the conclusion of the study and the indications for hysterectomy, one of them actually caused us to initiate the study, to see how often we had confused metaplasia with carcinoma or how often we had misdiagnosed it. This was an asymptomatic woman who had an endometrial biopsy specimen with ciliated and eosinophilic metaplasia. A curettage produced the same results, and the hysterectomy specimen showed a well-differentiated invasive adenocarcinoma. However, there was no real hyperplasia in the

biopsy specimen or the curettings. The second patient had hyperplasia with a great deal of metaplastic change, and metaplasia was the dominating feature that caught our attention. The lesion was designated as hyperplasia and metaplasia, then an invasive lesion was found at hysterectomy. I do not really have a good theory as to why we missed these lesions. We have looked back at the material, of course, in great detail, and on review the diagnosis was confirmed. The pitfalls, I think, are intellectual ones in that we believe that metaplasias in this age group and in this clinical setting are harmless. One of the articles we referred to said that metaplasias are of absolutely no clinical significance unless they are a source of confusion for the unwary pathologist. I think that even wary pathologists can be confused by this area, which is not so simple. I think it may be simple to talk about but hard to recognize. To me that is the pitfall. As far as prospective data, no we do not have that information. We have just completed this review of all of our cases of endometrial cancer. We are trying to plot out the future and we have not finished sorting the past or organizing it.

Conservative surgical management of isthmic ectopic pregnancies

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During the 12-month study interval ending March 30, 1986, there were 203 ectopic pregnancies at Grady Memorial Hospital, a ratio of one ectopic gestation per 34 deliveries. Twenty patients with isthmic ectopic pregnancies were selected for treatment by one of three operative modalities. Seven patients with ruptured isthmic ectopic pregnancies underwent segmental tubal resection without reanastomosis. All four patients who underwent segmental tubal resection with primary microsurgical reanastomosis had postoperative hysterosalpingograms demonstrating bilateral tubal patency. One pregnancy has occurred in this group. Nine patients underwent linear salpingostomy. In five of the six patients who had postoperative hysterosalpingography, patency of the involved fallopian tube was demonstrated. Four of these nine patients, including one patient with contralateral tubal occlusion, have conceived. We conclude that linear salpingostomy for isthmic ectopic pregnancies is as effective as segmental tubal resection with primary microsurgical reanastomosis in achieving tubal patency. (Am J Obstet Gynecol 1987;157:604-10.)

Key words: Isthmic ectopic pregnancies. linear salpingostomy, primary microsurgical reanastomosis

Linear salpingostomy has recently become the surgical treatment of choice in the management of ampullary or infundibular ectopic pregnancies, especially when future fertility is desired.[1-5] However, the optimum surgical approach to isthmic ectopic pregnancies remains controversial. Three conservative operations have been described: (1) segmental resection of the involved portion of fallopian tube and reanastomosis at a later operation, (2) segmental resection and primary microsurgical reanastomosis, and (3) linear salpingostomy.[1-9]

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Segmental resection with microsurgical reanastomosis at a later operation is the most favored technique.[5-8] Objections to primary reanastomosis include the technical difficulties in performing reanastomoses at an untimely hour, the variable skills of the surgeons involved in performing microsurgery, the additional operating time required, and the difficulty in assessing patency of the other fallopian tube during operation. However, excision of the isthmic ectopic pregnancy and primary reanastomosis without the aid of the operating microscope or loupes have been described by Stangel et al.[9] They have successfully performed this operation on seven patients, two of whom later had intrauterine pregnancies.[7, 9]

Linear salpingostomy for isthmic ectopic pregnancies is seldom described in the literature and is generally disfavored.[5-8] The narrow isthmic lumen may be particularly susceptible to ectopic implantation if lin-

ear salpingostomy results in additional scar formation within the fallopian tube. In a recent report, DeCherney and Boyers[8] concluded that simple linear salpingostomy without resection of the damaged portion of fallopian tube usually resulted in tubal obstruction.

The purpose of this study was to compare the success of linear salpingostomy with the success of segmental resection followed by primary intraoperative reanastomosis in achieving tubal patency.

Material and methods

Criteria for enrollment in the study included: (1) a preoperative diagnosis of ectopic pregnancy, suggested by an abnormal rise in quantitative determinations of human chorionic gonadotropin, ultrasound, and/or culdocentesis; (2) stable vital signs and hematocrit; (3) expressed desire by the patient for future fertility; and (4) intraoperative identification of an isthmic ectopic pregnancy. Informed consent was obtained before operation. When patients were found intraoperatively to have unruptured isthmic ectopic pregnancies, the involved fallopian tubes were alternately treated by linear salpingostomy or by segmental resection with primary microsurgical reanastomosis. In other words, unless a tubal microsurgeon was unavailable during the laparotomy, every other patient found to have an unruptured isthmic ectopic pregnancy underwent segmental resection followed by primary microsurgical reanastomosis, and the procedure to be performed was determined preoperatively. Patients found intraoperatively to have ruptured isthmic ectopic pregnancies were managed by segmental resection of the involved portion of the fallopian tube. Depending on the results of postoperative hysterosalpingography, these patients were candidates for reanastomosis at a second operation.

Segmental resection and primary reanastomosis were performed by a technique similar to that described by Stangel et al.,[9] with the exceptions that the operating microscope and loupes were used and reanastomosis was performed in two layers. A small Babcock clamp was placed on either side of the ectopic gestation to elevate and stabilize the fallopian tube. A 6-0 delayed absorbable suture was placed in the mesosalpinx directly beneath the ectopic gestation. The segment of fallopian tube containing the ectopic gestation was excised, and the suture was tied. The cervix was then occluded with a Buxton clamp and transfundal chromotubation was performed to document patency of the proximal portion of the fallopian tube. Retrograde chromotubation was performed to confirm patency of the distal segment of the fallopian tube. Microcautery was used to control small bleeding vessels and lactated Ringer's solution was used for continuous irrigation.[10]

Table I. Ectopic pregnancies at Grady Memorial Hospital between April 1, 1985, and March 30, 1986

	Range	*No.*	*%*	*Mean*
Age	15-39	202		25.7
15-19		17	8.4	
20-29		144	71.3	
30-39		41	20.3	
Parity	0-5	200*		
0		60	30.0	
1		77	38.5	
>1		63	31.5	
Gravidity	1-7	200*		
1		25	12.5	
>1		175	87.5	

*Gravidity and parity are unknown for two patients.

Microsurgical tubal reanastomosis was performed with interrupted 8-0 nylon suture in two layers.

Linear salpingostomy was performed in the same manner as described by others.[2-6] A linear incision was made along the antimesenteric border of the fallopian tube with the pinpoint tip of the Bovie electrocautery unit, and the ectopic pregnancy was gently delivered through this incision, with care taken to remove most of the trophoblastic tissue without denuding the placental bed and causing unnecessary bleeding. Hemostatis was achieved by using the bipolar electrocautery unit. The linear incision was left open.

Regardless of the operation performed, the pelvic cavity was thoroughly irrigated with lactated Ringer's solution. When laparotomy pads were necessary for exposure, they were moistened and placed in sterile plastic bags to avoid damage to serosal surfaces. Perioperative antibiotic prophylaxis was used routinely. Approximatley 200 ml of dextran 70 was placed into the peritoneal cavity before closure.[2] Postoperative hydrotubation was not performed, since its benefits have not been substantiated.[11] Hysterosalpingography was performed approximately 3 months after operation to evaluate tubal patency.

Results

The diagnosis of ectopic pregnancy was confirmed by laparotomy or laparoscopy in 202 patients at Grady Memorial Hospital during the 12-month study interval ending March 30, 1986. Grady Memorial Hospital serves the indigent patients of metropolitan Atlanta, Georgia. There were 6802 deliveries during that interval. Thus the incidence of ectopic pregnancies was 1:34 deliveries. This is the highest incidence of ectopic pregnancy reported in the American literature.

The ages of these patients ranged from 15 to 39, and the mean age was 25.7. The vast majority of patients were between 20 and 29 years of age (Table I). Parity

Table II. Location of ectopic gestation and condition of tube

Location of ectopic pregnancy	Condition of tube		No.	%
	Ruptured	Unruptured		
Ampullary or infundibular	69	71	140	69.0
Isthmicoampullary	1	5	6	0.3
Isthmic	23	17	40	19.7
Interstitial	13	2	15	7.4
Ovarian		1	1	<0.1
Abdominal			1	<0.1
Total No. of ectopic pregnancies			203*	
Total No. of patients			202	

*One patient had bilateral ectopic pregnancies.

Table III. Group 1: Patients with unruptured isthmic ectopic pregnancies who were treated by segmented resection and primary microsurgical reanastomosis

Patient	Age (yr)	Gravidity	Parity	Appearance of involved tube	Patency by chromotubation	Hysterosalpingography		Pregnancies
						Involved tube	Opposite tube	
D. R.	28	1	0	Within normal limits	Patent	Patent	Patent	Using contraception
D. R.	24	4	1	Within normal limits	Occluded	Patent	Patent	Using contraception
J. W.	19	3	1	Within normal limits	Occluded	Patent	Patent	Intrauterine pregnancy*
S. H.	32	2	0	Hydrosalpinx†	Occluded	Patent	Patent	No

*Documented by ultrasound.

†Fimbrioplasty performed intraoperatively, following which the tube was patent.

ranged from 0 to 5, and the mean parity was 1.2. The importance of future fertility for these patients is exemplified by the fact that 136 of these patients (68.5%) had one or no living children, and 60 patients (30%) were nulliparous. During their hospitalizations, 125 patients (62%) expressed a desire for future fertility and preferred tubal conservation if this could be done safely.

The ectopic pregnancy was located in the ampullary or infundibular portion of the fallopian tube in 140 patients (69%), in the isthmicoampullary portion in six patients (0.3%), in the isthmic portion in 40 patients (19.7%), and in the interstitial portion in 15 patients (7.4%) (Table II). One ovarian pregnancy and one abdominal pregnancy occurred. Among the 202 patients, there were 203 ectopic gestations; one patient was found to have bilateral tubal pregnancies. The incidence of repeat ectopic pregnancy for these patients was 7.4% (15 patients); two patients had repeat ectopic pregnancies within the study interval.

Twenty patients with isthmic ectopic pregnancies were enrolled in the study. They were divided into three groups: Group 1, patients with unruptured isthmic ectopic pregnancies managed by segmental resection and primary microsurgical reanastomosis; Group 2, patients with unruptured isthmic ectopic pregnancies managed by linear salpingostomy; Group 3, patients with ruptured isthmic ectopic pregnancies managed by segmental resection without reanastomosis.

Group 1 (Table III) consists of four patients with unruptured isthmic ectopic pregnancies who underwent segmental tubal resection and primary microsurgical tubal reanastomosis. Although three of these patients appeared to have tubal occlusion of the other fallopian tube by intraoperative transfundal chromotubation, postoperative hysterosalpingography demonstrated bilateral tubal patency. One of these patients now has an intrauterine pregnancy documented by ultrasound, and two are actively using contraception.

Group 2 (Table IV) consists of nine patients who underwent linear salgingostomy for unruptured isthmic ectopic pregnancies. The last three patients in this group underwent linear salpingostomy because a microsurgeon was not available at the time of laparotomy. Hysterosalpingography were performed in six of these patients, and five had patency of the involved fallopian tube. The remaining patient had a hydrosalpinx on the involved side, although the isthmic portion was patent. Four of these nine patients currently have intrauterine pregnancies documented by ultrasound or have already been delivered of their infants. One of these four pa-

Table IV. Group 2: Patients with unruptured isthmic ectopic pregnancies who were treated by linear salpingostomy

Patient	Age (yr)	Gravidity	Parity	Appearance of opposite tube	Patency by chromotubation	Hysterosalpingography		Pregnancies
						Involved tube	Opposite tube	
S. Y.	24	3	1	Within normal limits		Patent	Occluded	Delivery by cesarean section
B. W.	23	2	0	Within normal limits				Normal spontaneous vaginal delivery
D. J.	33	3	2	Within normal limits	Occluded	Patent	Patent	Intrauterine pregnancy*
J. B.	23	1	0	Within normal limits		Patent	Patent	Using contraception
S. E.	26	3	0	Within normal limits	Patent			Using contraception
M. Z.	22	2	0	Within normal limits				Intrauterine pregnancy*
S. G.†	24	2	0	Within normal limits		Patent	Patent	Using contraception
J. J.†	21	3	2	Within normal limits		Hydrosalpinx	Occluded	Using contraception
S. H.†	19	4	1	Within normal limits		Patent	Patent	Using contraception

*Documented by ultrasound.
†No microsurgeon available.

Table V. Group 3: Patients with ruptured isthmic ectopic pregnancies who were treated by segmental tubal resection

Patient	Age (yr)	Gravidity	Parity	Appearance of opposite tube	Patency by chromotubation	Hysterosalpingography		Pregnancies
						Involved tube	Opposite tube	
J. S. C.	26	2	1	Within normal limits		Occluded	Patent	Using contraception
S. D.	22	2	0	Within normal limits				Intrauterine pregnancy*
J. W.	22	3	2	Within normal limits		Occluded	Patent	Using contraception
V. M.	19	4	1	Hydrosalpinx†	Patent after fimbrioplasty	Occluded	Patent	Using contraception
D. C.	23	6	1	Within normal limits				
P. D.	31	4	0	Within normal limits	Occluded	Occluded	Patent	No
Y. J.	22	2	1	Within normal limits				Intrauterine pregnancy*

*Documented by ultrasound.
†Fimbrioplasty performed intraoperatively, following which the tube was patent.

tients, S. Y., demonstrated patency of the involved fallopian tube, but the opposite tube was occluded. Linear salpingostomy for this patient resulted in tubal patency followed by a successful intrauterine pregnancy. Two of these four patients have not had follow-up hysterosalpingography because they became pregnant before the procedure could be performed, and the remaining patient had bilateral tubal patency. The other five patients in this group are actively using contraception.

Group 3 (Table V) consists of seven patients who had ruptured isthmic ectopic pregnancies. Each of these patients were treated by segmental resection of the involved segment without primary reanastomosis. Four of these patients had postoperative hysterosalpingography that demonstrated patency of the opposite fallopian tube and, as expected, occlusion of the involved tube. Interestingly, one of these patients appeared to have an occluded opposite fallopian tube by intraoperative chromotubation. There are two pregnancies in this group, both occurring before hysterosalpingography could be performed.

Comment

The incidence of ectopic pregnancy is increasing.[12-14] An unpublished study at Grady Memorial Hospital in Atlanta revealed that the incidence of ectopic pregnancies between July 1, 1983, and March 30, 1985, was an alarming 1:44 deliveries (McSwain HF, Mitchell DE. Unpublished data). The subsequent year, the incidence of ectopic pregnancy at Grady Memorial Hospital was 1:34 deliveries, a 29% increase in the inci-

dence of ectopic pregnancies in a single year. The incidence of ectopic pregnancies per total pregnancies (deliveries and abortions) was subsequently compared. In spite of the rising number of abortions, a 27% increase in the incidence of ectopic pregnancies per total pregnancies was found.

After tubal pregnancy, 60% to 70% of patients are infertile.[14] In this series, 30% were nulliparous, and more than 60% wanted another child. Conservative operations with preservation of the fallopian tube are especially designed for the nulliparous woman and the patient with a history of infertility, for whom the removal of the involved fallopian tube may result in permanent sterility.

Isthmic ectopic pregnancies are uncommon, ranging from 5% to 15%[8] of all ectopic pregnancies. The percent of isthmic ectopic pregnancies at Grady Memorial Hospital in the past 12 months was 19.7. Because isthmic gestations are less common, it is almost impossible for anyone to determine the most feasible surgical approach. The high incidence of ectopic pregnancies at Grady Memorial Hospital made possible a comparison of these operative techniques, but even this series is small.

Linear salpingostomy has been successful in maintaining reproductive function in patients with ampullary ectopic pregnancies, possibly because the trophoblastic proliferation occurs mainly beneath the tubal mucosa.[15] This study indicates that linear salpingostomy may be successfully used for isthmic ectopic pregnancies, as well. Because isthmic ectopic pregnancies rupture at an earlier gestational age than ampullary ectopic pregnancies, the diagnosis must be made at the earliest possible time in order for a conservative operation to be performed. A high index of suspicion must be maintained. When the diagnosis of ectopic pregnancy is suspected, culdocentesis, serial quantitative human chorionic gonadotropin, ultrasonography, and diagnostic laparoscopy may confirm the diagnosis before irreparable tubal damage has occurred.

Stangel et al.[7, 9] have previously demonstrated that primary microsurgical reanastomosis after isthmic ectopic resection results in tubal patency and subsequent fertility. We have demonstrated that linear salpingostomy also results in tubal patency for isthmic ectopic gestations.

Is primary microsurgical reanastomosis necessary if the uninvolved fallopian tube is patent? Although reanastomosis has been performed in some series when the other fallopian tube was patent by hysterosalpingography, a controlled study is needed to determine if this procedure is indicated. In this series, intraoperative chromotubation was inconclusive in evaluating patency of the other fallopian tube. Intraoperative microsurgical reanastomosis may eliminate the need for a second operation.

Primary microsurgical reanastomosis requires the presence of a gynecologist with microsurgical capabilities. Linear salpingostomy may be performed successfully by any gynecologist who follows the basic operative principles practiced by most microsurgeons. In this series, linear salpingostomy, a conservative operation usually reserved for ampullary ectopic pregnancies, was as effective as primary microsurgical reanastomosis in achieving tubal patency.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. R. Montague Laffitte, Jr., Greenville, South Carolina (Official Guest). Over the last 20 years, there has been an increase in the incidence of tubal gestation. This is probably associated with the increase in the incidence of pelvic inflammatory disease, the use

of intrauterine contraceptive devices, and the increase of reconstructive tubal surgery. Early and aggressive antibiotic therapy for pelvic infections may also play a role by preventing complete occlusion of diseased tubes. During this same time, new and improved diagnostic tools (β-subunit of human chorionic gonadotropin, ultrasonography, laparoscopy), have led to earlier diagnosis of tubal pregnancy. This earlier diagnosis permits the use of conservative surgery in the treatment of this condition.

Conservative surgery should be considered in any woman who has a tubal pregnancy and who wishes to conserve her fertility, even in those cases with a normal contralateral tube. We have come a long way since Tait[1] described salpingectomy as definitive treatment for ruptured tubal pregnancies in 1884. In 1953, Stromme[2] became the first to describe a conservative approach to tubal pregnancy by performing a salpingostomy. Since then, many authors have reported on segmental excision of tubal pregnancies with or without anastomosis. More recently, Gomel,[3] DeCherney et al.,[4] and others have been strong advocates of the use of laparoscopy in the conservative treatment of this condition. Most authors conclude that there is no difference in the subsequent extrauterine pregnancy rates, whether the patients are treated conservatively or radically for the initial ectopic pregnancy, but there does seem to be an increase in subsequent viable pregnancy rates in those treated conservatively.

The study of Smith et al. has used a very well-defined subset of patients to compare the effectiveness of linear salpingostomy and segmental tubal resection with primary microsurgical reanastomosis in the conservative treatment of tubal pregnancy. Because of the low incidence of isthmic ectopic pregnancies, the numbers are small (N = 20).

Four patients with unruptured isthmic pregnancies underwent tubal resection and primary microsurgical reanastomosis. Postoperative hysterosalpingography demonstrated bilateral tubal patency. One of two patients has achieved an intrauterine pregnancy (two are actively using contraception). Stangel and Gomel[5] used this procedure in seven patients with isthmic pregnancy. The oviducts remained patent after operation in all seven. Two patients have become pregnant and were delivered uneventfully. In these two patients, the reconstructed tube was the only functional tube.

The second group of patients (N = 9) had unruptured isthmic ectopic pregnancies managed by linear salpingostomy, the technique that has been used quite successfully by DeCherney et al.[6] and others in the management of ampullary ectopic pregnancies. Five of six patients had patent tubes after operation. Two became pregnant before hysterosalpingography could be performed. Four of these nine have had documented intrauterine pregnancies. These numbers are more promising than the ones reported by DeCherney and Boyer[7] in 1985. In their study of 10 patients with isthmic ectopic pregnancies, the four treated by linear salpingostomy have not conceived. Hysterosalpingography demonstrated tubal occlusion in three of these four patients.

The third group consisted of seven patients with ruptured isthmic ectopic pregnancies treated by segmental resection without primary reanastomosis. Four of these patients had postoperative hysterosalpingography that revealed the opposite tube to be patent. Three of these four patients are now using contraception. Two of the seven patients have had intrauterine pregnancies (neither of these patients had postoperative hysterosalpingography).

From Dr. Smith's data and a review of the literature, it seems that segmental resection with either immediate or delayed anastomosis appears preferable to linear salpingostomy in the conservative management of unruptured isthmic pregnancies. Considering the anatomy of the fallopian tube, this is not surprising. The isthmus contains the narrowest lumen and the thickest musculature. Budowick et al.[8] have shown that the growing gestation rapidly penetrates the wall of the tube with the trophoblast destroying the surrounding muscularis. Segmental resection removes the damaged isthmus and the thick muscularis and simple endothelium facilitates anastomosis. Linear salpingostomy without resection of the damaged portion would seemingly result in isthmic obstruction.

In conclusion, conservative operation for ectopic pregnancies should be considered in any patient desiring to preserve her fertility. Early diagnosis is imperative; patients who have an unruptured tubal gestation and who are surgically stable are the best candidates for conservative therapy. Linear salpingostomy or tubal abortions seem to be the treatment of choice for ampullary ectopic pregnancies. Segmental resection, with or without anastomosis, seems to be the treatment of choice for isthmic ectopic pregnancies. However, Dr. Smith has shown that linear salpingostomy is effective in the treatment of isthmic ectopic pregnancies. The data are encouraging, and it would be interesting to look at these numbers again in 1 to 2 years and compare the results. Linear salpingostomy does offer several advantages. For the gynecologist with little or no training in microsurgery it is technically easier, and for this same physician it undoubtedly decreases operative time. One thing not mentioned but that must be considered by all gynecologists performing conservative operations is the problem with delayed hemorrhage. This can be secondary to persistent growth of gestational tissue. It has been recommended by Rivlin[9] that routine serial tests of the level of the β-subunit of human chorionic gonadotropin be done in the postoperative period.

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Dr. Lynn Gordon Borchert, Greenville, North Carolina. Dr. Smith, in my personal experience in dealing with unruptured ectopic pregnancy, I have used the microsurgical technique for linear salpingostomy in these patients and apparently in your series macrosurgical procedures were used exclusively. Has there been any study in your program comparing microsurgical linear salpingostomy to macrosurgical procedure in an attempt to identify potential bleeding sites and to coagulate them and occlude them before beginning the linear salpingostomy procedure? Along those lines what has been the interoperative morbidity of your patients who have had linear salpingostomy relative to blood replacement and hemorrhage?

Dr. Jaroslav F. Hulka, Chapel Hill, North Carolina. I have not a question but a comment. In this era of in vitro fertilization it becomes quite important to try to document the success of all sorts of fertility surgery as opposed to the 10% to perhaps 20% success of in vitro fertilization. Dr. Smith and colleagues are to be congratulated on beginning this in this rare problem and I hope they will continue to find some operations that will still be required or recommended rather than assuming that patients with these disasters should be automatically undergoing in vitro fertilization.

Dr. Smith (Closing). No one at our institution has attempted to use microsurgical techniques solely for linear salpingostomies. I have used loupes in performing this procedure. I have personally found that when I use the pinpoint tip of the Bovie instrument on low power I am coagulating most of the vessels. I personally have had a great deal of difficulty with engorged tubes in being able to obtain hemostasis with the bipolar cautery or microcautery. Several people at our institution, instead of using the pinpoint Bovie, were using a No. 15 blade or microscissors and they experienced more trouble bleeding than I have personally experienced when scrubbing with residents who were having to do a resection because of this. Those who required transfusion did not have either a microsurgical reanastomosis or a linear salpingostomy. It was felt that the condition of these patients was unstable and the most rapid technique should be performed. I did not include this in the article just to shorten its length, but we did look at operative times for each procedure. It is interesting to note than an average of only about 35 minutes more was required to do a microsurgical reanastomosis than a linear salpingostomy. A great deal of time should be taken to methodically make sure that the bleeding is controlled, so additional operating time is not really a contraindication to microsurgical reanastomosis.

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Simultaneous objective and subjective evaluation of meclofenamate sodium in the treatment of primary dysmenorrhea

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Eighteen patients participated in a double-blind, placebo-controlled, single-dose, crossover study of meclofenamate sodium in women with primary dysmenorrhea. Simultaneous evaluations of pain intensity and pain relief, sampling of continuous intrauterine pressure recording, and monitoring of blood meclofenamate levels were carried out. Improvements in pain intensity and pain relief were observed at 45 minutes and reached statistical significance at and beyond 1 hour 45 minutes after meclofenamate therapy. Ten of 14 uterine pressure parameters showed statistically significant responses after drug therapy and 12 of the 14 parameters showed statistically significant differences in time-response patterns. Statistically significant changes were noted as early as 45 minutes after meclofenamate therapy. Statistically significant correlations were found between and among the parameters of blood drug level and the subjective and objective measures. No drug-related adverse effects were found. (Am J Obstet Gynecol 1987;157:611-8.)

Key words: Dysmenorrhea, dysmenorrhea therapy, intrauterine pressure, meclofenamate, blood drug levels

Many studies have reported symptom improvement in patients with primary dysmenorrhea treated with nonsteroidal anti-inflammatory drugs. While success of treatment has been reported to vary from a low of 64%[1] to more than 95%,[2] results are affected by large variations in the data and relatively high placebo response rates found with pain evaluation. Unfortunately, only a small number of objective studies have been published, and these report on small study groups that vary from 3 to 13 patients.[3,4] None have combined the methodology of subjective assessment techniques with the rigor of objective measurements. Furthermore, to our knowledge, no study has attempted to correlate uterine activity with pain severity and each with blood drug level. To further understand the association of pain, intrauterine pressure, and plasma drug levels in patients with primary dysmenorrhea, the following investigation with meclofenamate sodium (Meclomen) was undertaken.

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Presented by invitation at the Forty-ninth Annual Meeting of The South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 25-28, 1987.
Reprint requests: Roger P. Smith, M.D., Department of Obstetrics and Gynecology, Section of General Gynecology, The Medical College of Georgia, Augusta, GA 30912.

Material and methods

A double-blind, placebo-controlled, single-dose, crossover study of subjective and objective responses to meclofenamate was undertaken in 18 patients with primary dysmenorrhea. Diagnosis was established on the basis of clinical history and physical examination. In addition, patients were required to have either a positive total score on a menstrual symptom questionnaire (which has been used in previous studies[4,5] and described elsewhere[6]) or to have had sufficient response to previous treatment with a nonsteroidal anti-inflammatory drug to have continued therapy. All patients had regular menstrual cycles, dysmenorrhea for more than 6 months' duration, and consistently moderate to severe symptoms without treatment. None of the patients were using oral contraceptives or an intrauterine contraceptive device or had contraindications to the use of nonsteroidal anti-inflammatory drugs. All patients were informed regarding the nature, procedures, and requirements of the study. Informed consent was obtained before entry into the study.

The study was conducted during two menstrual cycles, one while the patient received 200 mg of meclofenamate and one during administration of placebo, with the sequence determined by double-blind randomization. Each patient was asked to report to the clinic for study within 24 to 36 hours of the onset of menstrual flow and to take no medication within 6 hours of the study time. At the time of study, a catheter-tip microtransducer (Millar PC771) was placed

Table I. Data schedule

Monitoring dose	*Baseline**	*Time after treatment (min)*							
		15	*30*	*45*	*75*	*105*	*135*	*165*	*195*
Pain assessment	X	X	X	X	X	X	X	X	X
Intrauterine pressure†	X	X	X	X	X	X	X	X	X
Plasma drug level	X	X	X	X		X		X	

*Within 30 minutes before treatment; intrauterine pressure averaged over two 15-minute segments.
†Pressure recording for 15-minute segment ending at time interval.

to allow recording of intrauterine and endocervical pressures. Placement and recording were done according to previously reported methods.[7] A 21-gauge heparin lock was placed for blood sampling. A minimum of 30 minutes of baseline pressure recording was obtained, baseline subjective pain intensity was recorded, and a control blood sample was collected. The double-blind study medication was then administered.

Intrauterine pressure was continuously recorded for 3 hours 15 minutes after drug administration. Subjective and objective assessments and blood samples were obtained as shown in Table I.

Subjective analysis. Pain intensity was recorded as 0 = none, 1 = mild, 2 = moderate, or 3 = severe. Pain relief was recorded as 0 = none, 1 = a little, 2 = some, 3 = a lot, or 4 = complete.

Pressure analysis. Each pressure recording strip was divided into 15-minute segments for analysis. The two 15-minute segments preceding medication were averaged to provide baseline measurements. Response to medication was evaluated for the 15-minute segments ending as shown in Table I. Pressure recordings for each segment were converted into digital information and processed by microcomputer by means of a previously reported technique and algorithm.[7,8]

Computer analysis yielded 14 manometry parameters. For purposes of clinical evaluation, the parameters were categorized into general descriptive measures (average pressure, total area under the pressure curve, maximum rate of pressure increase, maximum rate of pressure decrease, number of pressure sign [slope] changes), measures of uterine work (maximum pressure, average peak pressure, number of peaks [contractions], contraction work, linear displacement), and measures of uterine rest (baseline pressure, baseline area under the pressure curve, percent time at baseline, and minimum pressure).

These parameters have been found to yield consistent and reliable characterizations of the pressure records.[7,8]

Meclofenamate blood level analysis. Plasma samples were analyzed at Warner-Lambert/Parke-Davis Pharmaceutical Research Division by means of a highly linear, specific, and stable high-power liquid chromatography assay.[9] The minimum detectable concentration of meclofenamate is 0.03 μg/ml.

Statistical methods. Two overall measures of pain were calculated for each subject at each cycle: the total of the pain relief scores (TOTPAR) and the sum of the pain intensity difference scores (SPID). TOTPAR and SPID were evaluated by a crossover analysis of variance that tested for treatment (drug) differences. In addition, the pain relief and pain intensity difference scores were analyzed by a crossover analysis of variance at each study time. The overall frequency of pain relief and pain intensity scores were tabulated and a χ^2 test was used to detect treatment differences.

Manometry parameters were analyzed for each subject at each cycle with the average of the pretreatment values used as the baseline response. The percentage of change from baseline was calculated for each of the eight posttreatment study times by the formula:

$$\frac{(\text{posttreatment response} - \text{baseline response})}{\text{baseline reponse}} \times 100$$

Comparability of baseline values across treatment groups for each manometry parameter were tested by a crossover analysis of variance. Percentage of change from baseline for each manometry parameter was evaluated using the analysis of variance for the two-period repeated measurements crossover design.* The fixed sources of variation were the period effects (cycle), direct treatment effects (drug), study time, and the interaction of study time with period and treatment. Three error terms were used to test the fixed effects of period and treatment, study time, and interaction.

The effects of interest were the treatment effect (difference between drug and placebo in the levels of the time-response curves), the period effects (difference in the order of treatments), and the interaction of time with treatment (difference between drug and placebo in the shape of the time-response curves). When the

*Wallenstein S, Fisher AC. The analysis of the two-period repeated measurements crossover design with application to clinical trials. Biometrics 1977;33:261-9.

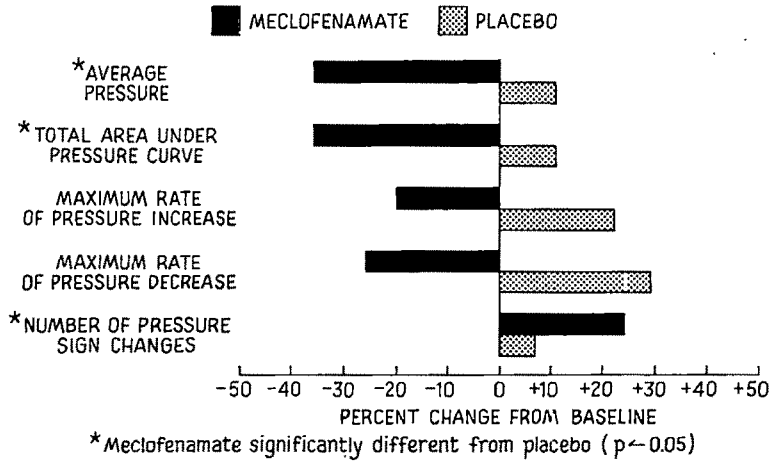


Fig. 1. Overall percent change from baseline—general descriptive measures.

interaction of time with treatment was significant at the 5% level, individual t tests were performed to compare drug response at each study time. These t tests were performed at an overall significance level of 5%.

Blood meclofenamate levels over time were analyzed by a repeated measures analysis of variance. Mean levels at each study time were compared by use of Duncan's multiple range test. The area under the curve was calculated by the trapezoidal method.

For the responses to meclofenamate treatment, Pearson product-moment correlation coefficients were calculated between meclofenamate blood levels and each of the pain parameters, between meclofenamate blood levels and the percentage of change in each of the manometry parameters, and between each of the pain parameters and the percentage change in each of the manometry parameters. Responses at the five time points at which meclofenamate blood levels were obtained were used. Corresponding correlations for responses on placebo (except blood level) were also computed. Correlation coefficients were calculated for each subject and the median coefficient was obtained. Each pairwise median correlation coefficient was tested to see if there was a correlation significantly different than zero.

All testing for statistical significance was performed at $p < 0.05$.

Results

A total of 18 patients entered the study. The overall mean age of the patients was 25.5 years (range 15 to 41). The average age of menarche was 12.0 years (range 9 to 13) and the average age at onset of dysmenorrhea was 15.1 years (range 9 to 39). Menstrual cycles were regular (mean = 28.9 days, range 21 to 35) and of average duration (mean = 5.4 days, range 4 to 8). Thirteen (72.2%) patients had never been pregnant, two (11.1%) had one pregnancy, and three (16.7%) had more than one pregnancy. Sixteen (88.9%) patients had taken one or more medications for dysmenorrhea within 3 months before the study. Entrance history and physical examinations on all patients were either normal or showed minor unrelated findings that would not interfere with the study.

All patients presented for study within 28 hours after the onset of menstruation (maximum = 27 hours; average time to meclofenamate therapy = 8.6 hours; average time to placebo therapy = 9.8 hours). No patients received any nonsteroidal anti-inflammatory drug within 102 hours of undergoing study.

Of the 18 patients entered, two patients were lost to follow-up after one cycle (placebo) and were not included in the crossover analyses of pain or uterine pressure parameters. One patient had a technically unsatisfactory pressure recording on one cycle (placebo) and was therefore excluded from the crossover analysis of uterine pressure parameters. All patients (excluding the one instance of unsatisfactory manometry measurements) were included in the calculation of means and correlation analyses. One patient experienced vomiting after taking study medication (placebo), and two patients received Compazine during the first hour of meclofenamate treatment for symptoms of nausea. These data were included in all analyses.

No patients experienced any drug-related adverse effects during this study.

Subjective response. Average pain intensity was moderate at baseline for both meclofenamate and placebo treatment groups. The differences between meclofenamate and placebo treatments were statistically significant for both total pain relief (TOTPAR) and sum of the pain intensity difference (SPID). The mean total of the pain relief scores was approximately 50% greater with meclofenamate (17.5) than with placebo (11.1), and the mean sum of the pain intensity difference scores was more than twice as great with meclofenamate

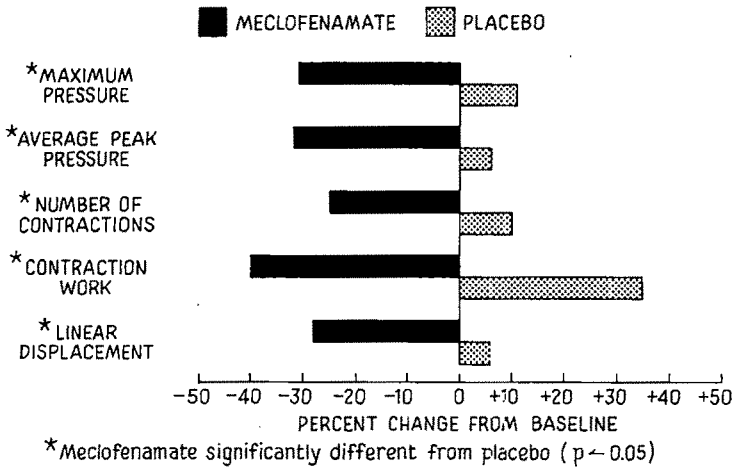


Fig. 2. Overall percent change from baseline—measures of uterine work.

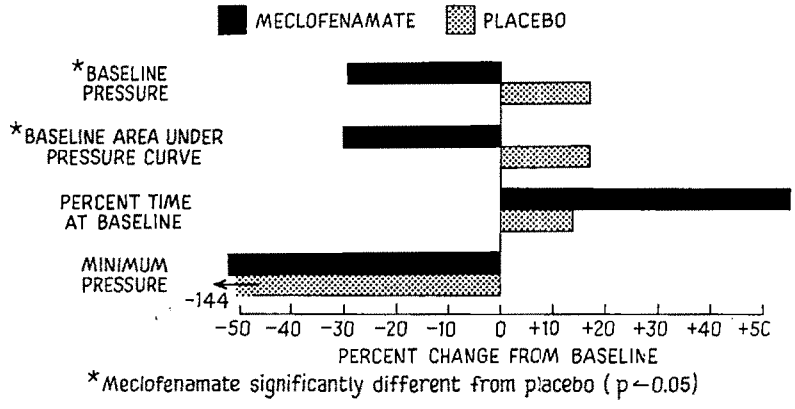


Fig. 3. Overall percent change from baseline—measures of uterine rest.

(7.6) than with placebo (3.4). The differences between meclofenamate and placebo treatments for both pain relief and pain intensity difference were evident as early as 45 minutes after treatment and reached statistical significance at and beyond 1 hour 45 minutes.

Objective response. The mean baseline values for each of the manometry parameters did not significantly differ by treatment group. The mean overall percentage of change from baseline was statistically significantly different by treatment group for 10 of the 14 manometry measures (Figs. 1 to 3). After meclofenamate treatment, a reduction from baseline of 20% to 56% was seen with all parameters except number of pressure sign changes and percent time at baseline, whereas after placebo treatment, an increase of 6% to 35% was seen with all parameters except minimum pressure.

The increase in number of pressure sign changes of 24% with meclofenamate versus 7% with placebo (Fig. 1) reflects an increase during meclofenamate treatment of small frequent pressure changes common in the resting uterus, as opposed to the prolonged rises and falls in pressure seen with untreated uterine contraction waves. Measures of uterine work showed reductions ranging from 25% to 40% after meclofenamate and increases of 6% to 35% after placebo. Five (31%) patients receiving meclofenamate had a complete loss of contractile activity on meclofenamate (number of peaks = 0). A reduction in number of peaks (contractions) to <20% of baseline was experienced by 10 (63%) patients receiving meclofenamate and only three (18%) patients receiving placebo. During placebo therapy, four (25%) patients experienced a twofold or greater increase in the number of uterine contractions as measured by number of pressure peaks.

Twelve of the 14 uterine pressure parameters showed statistically significant differences between meclofenamate and placebo in the shape of the time-response curves. These differences were apparent as early as 30 minutes after medication, were generally

evident at 45 minutes, and became statistically significant at 45 minutes to 1 hour 45 minutes after medication.

Plasma meclofenamate levels. No detectable plasma meclofenamate levels were present for any patient at baseline or after placebo administration. After administration of meclofenamate, mean plasma drug levels increased steadily during the study period, from 0.25 to 4.97 μg/ml. The greatest increase occurred between 15 and 30 minutes after treatment ($p < 0.05$).

Correlations between parameters. After meclofenamate administration, statistically significant correlations were found between blood meclofenamate level and pain relief, pain intensity difference, and percent change in all manometry parameters except contraction work and percent time at baseline. Statistically significant correlations were also found between pain relief and percent change in all manometry parameters except contraction work and between pain intensity difference and percent change in all manometry parameters except contraction work and percent time at baseline. The magnitude of the r values of statistically significant correlations ranged from 0.26 to 0.87.

After placebo administration, statistically significant correlations were found only for pain intensity difference and percent change in linear displacement ($r = -0.51$), and pain intensity difference and number of pressure sign changes ($r = 0.52$). Pain relief showed no statistically significant correlations with any of the manometry parameters during placebo.

Comment

Past studies have suggested that the pain of dysmenorrhea is directly related to the amount of uterine work (high pressures and number of contractions) and inversely related to the quality of uterine rest.[8, 10-14] In this study, simultaneous recording of pain and uterine activity after placebo and drug therapy was shown, for the first time, that a reduction in intrauterine pressure and uterine activity during drug treatment results in a reduction in pain associated with primary dysmenorrhea. Several findings clearly demonstrated the effectiveness of meclofenamate in treating primary dysmenorrhea: (1) statistically significant decreases in both intrauterine pressure and pain; (2) statistically significant correlations between pressure and pain measures during drug therapy; and (3) statistically significant correlations of pressure and pain measures with meclofenamate blood level.

Pain improvement was observed shortly after administration of both drug and placebo. However, this improvement reached a modest, sustained level of response soon after placebo, whereas progressive improvement was observed after meclofenamate. In fact, it appears that maximal effects were not yet reached by the end of 3 hours 15 minutes despite clinically and statistically significant changes much earlier. Comparisons of the meclofenamate and placebo response versus time curves for all pressure measures except minimum pressure do not reveal a similar "placebo effect" for objective responses.

The greatest changes in manometry measurements after meclofenamate therapy occurred in parameters of uterine work (Fig. 2). All parameters of uterine work showed statistical significance of meclofenamate over placebo, with significant differences occuring as early as 45 minutes after ingestion of the drug. Almost two thirds (63%) of the patients receiving meclofenamate experienced a reduction in the number of uterine contraction waves (pressure peaks) to below 20% of their pretreatment levels. By comparison, four (25%) patients receiving placebo experienced at least a twofold increase in the number of uterine contractions. Furthermore, the contractions occurring during meclofenamate therapy showed a 32% reduction from baseline in average peak pressure. The reductions in the frequency and severity of contractions after meclofenamate are reflected in an overall 40% reduction in contraction work, whereas after placebo, contraction work increased by 35%. Linear displacement as a measure of uterine work similarly showed a reduction of 28% with meclofenamate and an increase of 6% with placebo. The rapid reductions in uterine work after meclofenamate therapy and the statistically significant correlations of these reductions with blood meclofenamate level and pain improvement confirm the relationship of uterine work in the development of pain in dysmenorrhea, as suggested in a recently published review.[15]

The moderate increase in percent time at baseline observed for placebo (14%) occurred during a simultaneous increase in baseline pressure of 17%, while the greater increase observed for meclofenamate (56%) occurred simultaneously with a 29% reduction in baseline pressure. Thus patients receiving meclofenamate spent more time at a reduced resting pressure than did those receiving placebo.

Past discussions of the proposed relationship between the uterine activity recorded by objective means and subjective discomfort have been based on little more than clinical conjecture. The consistent correlations found in these data for meclofenamate treatment provide strong evidence that drug therapy directly affects the uterine activity that causes the discomfort of dysmenorrhea. Thus a claim of pain prevention, rather than pain relief, seems highly justifiable.

In the clinical setting, treatment effect may be even greater than that seen in this study. Patients in this study

had discomfort of at least moderate severity before study and then underwent at least 30 additional minutes of baseline uterine pressure recording. In clinical practice, it is recommended that medication be taken at the first indication of discomfort rather than after increased severity of symptoms. Thus meclofenamate may provide even greater therapeutic effect in dysmenorrheic patients treated when symptoms first appear.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. MICHAEL PHELAN, Jacksonville, Florida (Official Guest). The use of nonsteroidal anti-inflammatory agents for the treatment of dysmenorrhea is commonplace. For many years these drugs were used with no clear understanding of their mechanism of action. Previous studies have indicated that elevated myometrial tonus between contractions is an important factor in both primary and secondary dysmenorrhea. In fact, the basal tone of the uterus may be more of a factor than the rhythm or intensity of contractions in that the elevated uterine tone causes hypoxia of the uterine muscle and therefore pain.[1] Anti-inflammatory agents work by inhibition of prostaglandin synthesis and lowering of the resting tone of the uterus during menstruation.

Previous studies have demonstrated accurate methods of obtaining information and evaluating activity of the uterus during the menstrual period.[2] Drs. Smith and Powell used this technology to evaluate the drug meclofenamate and to show both the efficacy of the drug and the action of the drug on uterine activity. Their study is a double-blind, placebo-controlled, crossover study of 18 patients that compares both the subjective discomfort of dysmenorrhea and the effect of medication on uterine activity. We are shown simultaneous evaluation of pain intensity and pain relief, continuous recording of intrauterine pressures along with monitoring of blood meclofenamate levels. The significant correlations made are those of blood meclofenamate levels with pain relief parameters, blood levels, and reduction of uterine activity as well as diminished uterine activity and pain improvement. The conclusions drawn are that there is statistically significant relief achieved with meclofenamate and that the action can be directly correlated with blood drug levels and the degree of alteration in objective measurements of uterine activity.

Similar studies[3] with the use of intrauterine pressure monitoring and other medications have been done in the past, but none have combined the subjective and objective measurements that the authors used. Most of the comments that can be made on this study are general comments that have been made in regard to all these types of studies. The first and most obvious is the concern that the information gathered on a study group this size cannot be extrapolated to a much larger group, the general population. The fact is that investigators do not claim that their drug will relieve all patients of dysmenorrhea. All that needs to be shown is that the medication is significantly effective in the majority of patients using it when compared with a placebo group. In this study there was "a moderate increase in percent time at baseline uterine pressure during a simultaneous increase in baseline pressure of 17% for placebo." With meclofenamate there was a greater increase in percent time at baseline (56% versus 14%) with a 29% reduction in baseline pressure. Patients receiving meclofenamate spent more time at a reduced resting pressure and therefore experienced less pain.

Smith and Powell stated that there were no adverse effects of the drug, but two patients experienced nausea and were given Compazine. One patient receiving placebo vomited. One of the major drawbacks of using earlier anti-inflammatory agents such as indomethacin

or phenylbutazone was their gastrointestinal side effects. This has been thought to be due to the fact that there is a protective effect by prostaglandins on gastric mucosa.[4] Suppression of prostaglandins therefore allows for gastric upset. Concerns also have been raised regarding long-term use of anti-inflammatory agents. Critics often fail to note, however, that with dysmenorrhea the use of the medication is short term and thus the gastrointestinal effect may not be as significant. Only further experience with the drug will yield that information.

The use of nonsteroidal anti-inflammatory agents for treating secondary dysmenorrhea is also an issue. The fear that arises is that pain relief given by these medications may mask a progressive, damaging process such as endometriosis. These drugs do not give relief to all patients and a trial of drug therapy may actually help select out the patients that need to undergo laparoscopic examination. This article dealt with primary dysmenorrhea, but with endometriosis being found in younger patients this may become a growing concern.

There have been shown to be many effective nonsteroidal anti-inflammatory agents for treating dysmenorrhea, but the studies have been small and "head-to-head" comparisons of drugs are lacking. While there is probably no "best drug" for treating this problem it would be nice to know which medications would carry the highest expectations. Meclofenamate certainly has been shown to give significant relief to patients. It meets the criteria described by Henzl et al.[5] for the "ideal drug" for treatment of dysmenorrhea. It acts by direct interference with the pathogenic process, its use may be limited to the time when an individual suffers the pain, it is highly effective without undue side effects, and the onset of analgesia is prompt. Subjective responses to differences in pain relief and pain intensity were evident as early as 45 minutes after treatment with meclofenamate. The pain relief correlated well with blood drug levels.

In closing I would have to agree with the conclusion of Smith and Powell that "a claim of pain prevention, rather than pain relief, seems highly justifiable."

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Dr. William F. O'Brien, Tampa, Florida. My question is regarding the correlation with blood drug levels. It appeared to me that the measurements were taken at a time when blood levels were on the rise and therefore there was automatically an autocorrelation with time. In order to truly look at correlation one would then have to look at the down slope of that to see if the symptomatology and the objective findings correlated there rather than just with time after drug use. I would like a comment on that.

Dr. John Staegie, Durham, North Carolina. I am fascinated by the objective worsening of measures on the placebo. I am wondering if that is an artifact of the prolonged placement of the catheter device itself. If not, could he speculate on the mechanism for the subjective worsening in the presence of objective improvement.

Dr. Smith (Closing). Dr. Staegie, I think the reason that we see worsening over time in placebo-treated patients relates to the natural history of these diseases. We were lucky enough to be able to catch these patients fairly early into their symptoms and the natural course of these patients generally is a worsening over the first 24 to 48 hours. The average time from onset to presentation and start of the monitoring process for the placebo patients was around 8 hours. It was slightly longer for the meclofenamate patients but there was no statistically significant difference. Our feeling is that this is not catheter induced. When we have had an opportunity for long-term monitoring with the catheter we have seen no long-term changes. For example, in earlier techniques with the use of microballoons, many investigators felt that there had to be a 30-minute cooling off period from the time of insertion of the balloon until the time the readings were stable because there was an induction of uterine activity at that point. In our hands we have not seen any changes with placement of the catheter or with long-term use. Paralleling that is the first question about long-term monitoring and whether we see these drugs wear off again. While this study was limited to 3½ hours, the maximal blood effect did not appear to have been reached. In other patients in other studies you can indeed watch these drugs wear off. With monitoring a progressive return to baseline activity can be seen around 6 to 7 hours. Even though we did not make any effort to look at the wear-off period we feel confident that the rise and the parallel change in uterine activity still reflect the effect of our therapy. Whether we can undo that therapy is a separate question.

The question raised in the formal discussion about the response in secondary dysmenorrhea of course is a concern. Obviously any medication used for pain should not cover up the underlying cause. It has been our experience as well as that of other investigators that patients with secondary dysmenorrhea generally do not respond well to the nonsteroidal drugs. As Dr. Phelan alluded, this may almost be a therapeutic challenge. If a patient who is receiving a nonsteroidal drug of any sort does not get at least some improvement, I think you have to be suspicious that you have the wrong diagnosis. The patient has secondary dysmenorrhea. The question of nausea with these drugs and with dys-

menorrhea is a problem. In a larger subjective study of Ponstel, a cousin of meclofenamate, there was a one-half instance of nausea with drug as opposed to placebo. Many of your patients will tell you that a common part of their dysmenorrhea is nausea. This is thought to be due to the gut stimulation by prostaglandin $F_{2\alpha}$, a very potent smooth muscle stimulant; it makes sense that it should cause nausea, vomiting, and diarrhea through gut hypermotility. While we did see two patients in this study who had nausea sufficient to require an antiemetic and they were receiving meclofenamate, our feeling in this study was that it was not related to the drug; however, we do know that roughly 4% to 6% of patients receiving nonsteroidal drugs will have nausea. Dr. Phelan pointed out the significance of baseline activity in our hands, and in the actual correlation numbers, we felt that uterine activity was even more important in the cause of pain. The amplitude and the rate of change and the frequency of uterine contractions appear to be even more important than just baseline activity but more work still needs to be done.

Hysterosalpingographic evaluation of tubal patency after ectopic pregnancy

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In the period 1983 to 1985, hysterosalpingography was performed in 76 women after laparotomy to manage ectopic gestations. Clinical characteristics of these women, including their operative findings, were then correlated with the follow-up hysterosalpingography. Study participants were classified into five groups according to surgical management of the ectopic gestation. In each of the five groups, a substantial percentage of those women with a nonpatent fallopian tube (contralateral to the tube bearing the ectopic gestation) on hysterosalpingography had a normal-appearing fallopian tube at laparotomy. Approximately 50% of women with a history of previous pelvic inflammatory disease were found to have nonpatent contralateral fallopian tubes on follow-up hysterosalpingography. (Am J Obstet Gynecol 1987;157:618-22.)

Key words: Hysterosalpingogram, ectopic pregnancy, reproductive outcome after ectopic pregnancy

The dramatic increase in the incidence of ectopic pregnancy has created an increasingly important personal and public health problem. With so many women involved, a clear understanding of reproductive potential after ectopic pregnancy is necessary, yet relatively little information exists to assess such reproductive potential.

To better characterize factors associated with reproductive outcome after ectopic pregnancy, we undertook a prospective study of women undergoing operation for ectopic gestations at Grady Memorial Hospital during the period 1983 to 1985. We attempted to determine the relationship between clinical characteristics identifiable before operation, anatomic characteristics assessed intraoperatively, and postoperative tubal status as measured by hysterosalpingography.

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Material and methods

On July 1, 1983, a protocol was established on the Gynecology and Obstetrics Service at Grady Memorial Hospital, whereby all women who underwent tubal operation for an ectopic pregnancy between July 1, 1983, and March 31, 1985, were evaluated in the third postoperative week. At that time hysterosalpingography was scheduled for the proliferative phase of the menstrual cycle (days 7 to 11), with an effort being made to schedule the hysterosalpingography as closely as possible to the sixth postoperative week. Women had a Westgren sedimentation rate determined the day prior to the scheduled hysterosalpingography. If the sedimentation rate was elevated, the hysterosalpingogra-

Table I. Selected characteristics of women with ectopic pregnancy, Grady Memorial Hospital, 1983 to 1985

	No.	*%*
Age (yr)		
15-19	8	10
20-24	32	42
25-29	25	33
30-34	9	12
35+	2	3
Parity		
0	29	38
1	33	43
2	9	12
3+	5	7
History of IUD use		
No	47	70
Yes	20	30
History of PID		
No	40	60
Yes	27	40
History of previous ectopic gestation		
No	65	97
Yes	2	3

phy was rescheduled to follow a 10-day course of antibiotics. Women who did not keep follow-up appointments were contacted and encouraged to do so.

All women with ectopic pregnancy during the study period were considered for conservative surgical management. Salpingectomy was performed only if less conservative management was not clinically feasible. Partial salpingectomy was performed in all women with isthmic pregnancies. For ampullary pregnancies, ruptured or unruptured, an attempt was made to perform a linear salpingostomy when such management was considered clinically feasible. Partial salpingectomy was carried out if damage to the tube was extensive or bleeding was otherwise uncontrollable. Although surgical procedures were performed by various surgeons, all used microsurgical techniques without magnification. Adjuvant therapy included instillation of intraperitoneal dextran 70 (Hyskon), 200 ml, and intravenous chlorpromazine and dexamethasone after operation. A detailed description of the woman's pelvis was recorded intraoperatively on a form placed in her medical record for that purpose.

Our study population comprises those women who had a verified ectopic gestation at laparotomy and who completed postoperative follow-up with hysterosalpingography during the study period of July 1, 1983, to March 31, 1985. We attempted to obtain operative reports, descriptions of the pelvic anatomy, and reports on hysterosalpingography for all patients. History of previous pelvic inflammatory disease (PID), intrauterine contraceptive device (IUD) use, previous ectopic pregnancies, and subsequent intrauterine or ectopic pregnancies was obtained through chart review and telephone interviews.

Table II. Ectopic pregnancy study groups by surgical management, Grady Memorial Hospital, 1983 to 1985

Group No.	*Surgical management*	*No. of women*
1	Salpingectomy	26
2	Partial salpingectomy	26
3	Salpingostomy	13
4	Mechanical tubal abortion	5
5	Other	6
Total		76

Results

During the study period, 264 women underwent operation for ectopic pregnancies at Grady Memorial Hospital. During the same 21-month period, 11,792 deliveries took place. Thus at this hospital ectopic pregnancies occurred during our study in a ratio of 1:45 deliveries.

Seventy-six (29%) of these 264 women underwent postoperative hysterosalpingography. The ages of these 76 women ranged from 16 to 37 years. While gravidity ranged from 1 to 8, with 17 (22%) being primigravid, parity ranged from 0 to 7, with 29 (36%) of these being nulliparous (Table I).

In 67 women, we obtained history concerning previous PID and IUD use. Of these 67, 20 (30%) had a history of IUD use and 27 (40%) had a history of previous PID. Two women had previous ectopic pregnancies (Table I).

The study participants were classified into five groups according to the surgical management of the ectopic gestation (Table II). Group 1 was composed of the 26 women who underwent total salpingectomy. Postoperative hysterosalpingography showed that 16 of these women had contralateral tubal patency. In six of the 10 women with a nonpatent contralateral tube, these tubes appeared normal at laparotomy.

Group 2 consisted of 26 women who underwent a partial salpingectomy. In 21 of these 26, hysterosalpingography showed a patent contralateral tube. In two of the five women with a nonpatent contralateral tube, these tubes were described as normal at laparotomy.

Group 3 included 13 women managed by linear salpingostomy. On follow-up hysterosalpingography, four had bilateral tubal patency, six had unilateral patency only (four contralateral tubes, two tubes that were operated on), and three had bilateral occlusion. Of the nine women with at least one nonpatent tube, only four

Table III. Relationship of past IUD use and history of PID to hysterosalpingographic demonstration of contralateral tubal patency, Grady Memorial Hospital, 1983 to 1985

Result of hysterosalpingography	*No IUD use or PID*	*IUD use only*	*PID only*	*IUD use and PID*
Contralateral patency	22	9	8	5
Contralateral obstruction	9	0	8	6
Total	31	9	16	11

were described as having a normal contralateral tube at laparotomy. Two were described as having an abnormal contralateral tube with no description available for three women.

Group 4 consisted of five women who underwent tubal manipulation to induce mechanically abortion of distal tubal pregnancies. Two of these women had nearly complete spontaneous abortions. On follow-up hysterosalpingography, two of these women appeared to have bilateral patency, two had unilateral patency (one tube that was operated on, one contralateral tube), and one had bilateral obstruction. Of the two women with contralateral tubal obstruction on hysterosalpingography, one had a normal contralateral tube at laparotomy.

Group 5 consisted of six women who could not be categorized in any of the above groups. One had evacuation of an abdominal gestation. Two had previous tubal ligations and underwent salpingectomy. One had had two previous ectopic pregnancies and underwent salpingostomy. Another had bilateral tubal gestations managed by right partial salpingectomy and left salpingostomy. Of these six women, all had bilateral obstruction on hysterosalpingography.

We also evaluated, by follow-up hysterosalpingography, the effect of previous PID and IUD use on tubal patency after ectopic pregnancy in the 67 women for whom this information was available (Table III). There were 31 women with no history of previous IUD use or PID. Twenty-one (79.7%) of these had contralateral tubal patency on hysterosalpingography. There were nine women who had used an IUD but had not had PID. All of these had patent contralateral tubes. There were 16 women who had a history of PID but had not used an IUD. Eight (50.0%) had contralateral tubal patency. Eleven women had a history of both IUD use and PID and only five (45.5%) of these had contralateral tubal patency on hysterosalpingography. Six of the 11 women had ipsilateral as well as contralateral tubal obstruction. None of these women had bilateral patency on hysterosalpingography.

Comment

Conditions delaying or preventing the transport of the embryo cause most extrauterine pregnancies. Chronic salpingitis can impair ciliary activity and tubal motility.[1] Although some data suggest that past IUD use does not increase the risk of ectopic pregnancy,[2] two recent case-control studies[3, 4] demonstrate an increased risk of tubal infertility after IUD use, particularly when such use is associated with tubal infection. In our study, 30% of the study population had used an IUD in the past. Forty percent gave a history of previous PID. While we have no control group for comparison, we believe that this prevalence of IUD use and PID in our study population is higher than would be expected for all women receiving care at our institution.

We correlated a history of IUD use and PID with findings at postlaparotomy hysterosalpingography. All women with IUD use but no previous PID showed contralateral tubal patency on hysterosalpingography, while approximately 50% of those with only a history of PID and those with IUD use plus a history of PID showed contralateral patency. Thus a history of previous PID may be an important predictor not only of risk for ectopic pregnancy but also for subsequent tubal patency. IUD use alone did not appear to increase the risk of tubal obstruction in our population.

Patients having follow-up hysterosalpingography were self-selected and thus potentially introduced a selection bias. We have minimal information to compare characteristics of participants and nonparticipants in this regard. However, the minimal relevant demographic and clinical information available suggests no large difference in the two populations. Specifically, of the 188 women not participating in the study, we were able to identify the ages of 95 patients. Ages of these women ranged from 16 to 35 years, similar to the range for the study population. Few data were available to assess a history of PID or IUD use in those not participating in the study. Of the small numbers of records available for review, four of 14 (28%) and three of 15 (20%) indicated a history of PID and IUD use, respectively. These percentages are generally comparable to those of the study population.

Our protocol for managing ectopic pregnancy attempted to maximize subsequent fertility by use of conservative operation, with careful operative technique. One must ask if conservation of the tube bearing the ectopic gestation is in the patient's best interest. In

our patient population, many women with normal-appearing contralateral tubes at laparotomy had nonpatent contralateral tubes by hysterosalpingography. The fact that clinical assessment and inspection alone at the time of laparotomy for an ectopic pregnancy do not accurately correlate with findings at postoperative hysterosalpingography underscores the importance of conservation of fallopian tubes whenever possible. When conservative operation is performed, hysterosalpingography performed after operation may help to better assess the contralateral tubes and the tubes that were operated on. This information may be useful in counseling regarding future fertility and the need for additional surgical intervention.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Edward E. Gahres, Alexandria, Virginia (Official Guest). During the past decade, the incidence of ectopic pregnancy has doubled in most areas of this country. The study of Mitchell et al. has confirmed this fact. It has been reported that as many as 70% of the women who have had an ectopic pregnancy are infertile. The recurrence rate for ectopic pregnancy is at least 10%. Ectopic pregnancy is the fourth leading cause of death in the state of Virginia. The two leading causes of ectopic pregnancy appear to be salpingitis and previous IUD use. It is unfortunate that the presence or absence of salpingitis was not histologically reported in the surgical specimens studied in this study. Histologic evidence of previous salpingitis has been reported by others to vary from 25% to 50% in uterine tubes removed at the time of operation for ectopic pregnancy.

Since many women desire pregnancy after an ectopic pregnancy, some means of evaluating the status of the fallopian tube in these women is desirable so that they can be advised of their chances for successful pregnancy. Laparoscopy with tubal insufflation produced by a dye appears to be the most accurate means for predicting future success,[1] but it is costly and not entirely without dangers to the woman. Hysterosalpingography is easier, safer, and less expensive and may be as accurate as laparoscopy.

Of the five groups of women reported on in this study, the first four groups consisting of 70 women are the ones who might benefit from screening by hysterosalpingography. Of these 70 women, 51 were determined to have unilateral or bilateral tubal patency. It might be of benefit to follow up these women to ascertain how many have attempted pregnancy. The pregnancy rate might determine the usefulness of hysterosalpingography as a predictor of successful future pregnancy.

The finding that all the women with a history of IUD use but no previous history of PID had contralateral tubal patency is unusual. Knowing the type of IUD used might partially explain this fact, since Dalkon Shield users have an ectopic pregnancy rate that is sixfold higher than that of copper-bearing device users. It has been suggested that removing the IUD as a contraceptive will relegate organisms that produce actinomycosis to a nonpathogenic role and decrease the incidence of unilateral salpingitis.

The presence of *Chlamydia trachomatis* antibody has been reported to be indicative of past tubal disease. The testing for this antibody might be useful as an additional screen for predicting future successful pregnancy.

As Dr. Mitchell has indicated, this study may be useful in counseling women in regard to future fertility.

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Dr. Lynn Borchart, Greenville, South Carolina. Dr. Mitchell, have you observed the possible beneficial effect of the hysterosalpingogram itself in the subsequent pregnancy in comparing patients who have been treated for ectopic pregnancy in similar fashions?

Dr. Herbert G. Hopwood, Jr., Arlington, Virginia. I would like to ask Dr. Mitchell one question. Does she contemplate joining in the future the new avant-garde wave of starting the person with suspected ectopic pregnancy on a regimen of methotrexate? Has she planned to do any type of study of those patients?

Dr. Jaroslav F. Hulka, Chapel Hill, North Carolina. I enjoyed the fact that the IUD did not get a "bum rap" in causing ectopic pregnancy in this study, but my concern has to do with those patients that were not studied. A total of 79 patients underwent hysterosalpingography in a total of more than 240 patients. Could you comment at all about the characteristics of those that were not studied? Were the 79 representative of your population?

Dr. H. Oliver Williamson, Charleston, South Carolina. I would like to ask if the authors looked specifically for salpingitis isthmic a nodosa. We hear of salpingitis and IUD usage, but the occurrence of this entity, I would think, should be picked up in a series this large on histologic examination as well as on a hystero-

salpingogram. I did not see that. I would ask specifically did they note it?

Dr. Mitchell (Closing). Yes, there are going to be continuing studies. At this time we just wanted to follow up those individuals who had ectopic pregnancies in this particular group of 76 women. We have persistently followed them up because they are somewhat transient, and that also answers Dr. Hulka's question. We have had 30 intrauterine pregnancies in the 76 individuals, so we are continuing follow-up on these individuals and have had only three repeat ectopic pregnancies. We are very pleased with these results. I think that the most difficult problem we have is because our patient population at Grady Hospital is so transient. Most individuals do not have telephone numbers when we try to call 6 weeks later. They have moved or are just not available and that is very difficult for us, but these are individuals whom we have counseled as much as we can. It may be a point of a resident being able to convince them to come back to Grady Hospital or to come to Grady Hospital as their base more than some of the other 200 that we were unable to follow up. We would like to improve that and we would like to report on all the patients, but that is not possible in our patient population as I think you can imagine. As far as the kind of IUD, we do not have that information. Most of the time it is very nice for us just to know that they had an IUD, again considering the population. Right now we are in the process of doing some chlamydial testing at Grady Hospital, and I think that will become one of the main aspects we are hoping to correlate with future studies on ectopic gestations in our institution. As to methotrexate, yes, I would very much like to think about using that if we can start getting our patients in earlier. Our patient population also is associated with difficulty in coming in so early. Patient education has helped, but many of these individuals come in after rupture and obviously we would not be able to give them methotrexate. I do think that is the next thing we would like to do or at least think about. No, we do not look specifically for salpingitis isthmic a nodosa, but I think that if we continue to do these studies we will come up with a subgroup of those individuals. As far as the treatment that you just asked about, I think we will just have to look at the numbers that have had intrauterine pregnancies and divide up those groups. A lot of these women did get pregnant rather quickly afterward so that might go along with your question.

Transurethral ultrasound-guided oocyte retrieval for in vitro fertilization

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Thirty-nine follicular aspirations for in vitro fertilization were performed in 30 patients. Oocyte retrieval was performed by laparoscopy in 21 cases and ultrasound-guided transurethral aspiration in 18 cases. All procedures were performed by the same ovarian stimulation protocol and the same aspirating needle. In the laparoscopy group 150 oocytes were recovered from 177 follicles aspirated (85%). The fertilization rate was 69% (104/150) and cleavage rate, excluding triploidy, was 82% (85/104) for oocytes obtained by laparoscopy. Five clinical pregnancies occurred in the laparoscopy group for a pregnancy rate of 24% per cycle and 25% per transfer. In the transurethral ultrasound-guided group 77 oocytes were recovered from 103 follicles aspirated (75%). The fertilization rate was 79% (61/77) and cleavage rate, excluding triploidy, was 90% (55/61). Three pregnancies occurred for a pregnancy rate of 17% per cycle and 18% per transfer. None of these differences were statistically significant. Transurethral ultrasound-guided oocyte retrieval is a feasible alternative to laparoscopy for in vitro fertilization. (Am J Obstet Gynecol 1987;157:622-6.)

Key words: Ultrasound, fertilization, oocyte

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Laparoscopy is the usual method of oocyte retrieval for in vitro fertilization. Pregnancy rates per cycle range from 10% to 30% in most successful programs, indicating that the procedure may need to be repeated several times in many women. The least expensive and the least invasive procedure for oocyte retrieval should

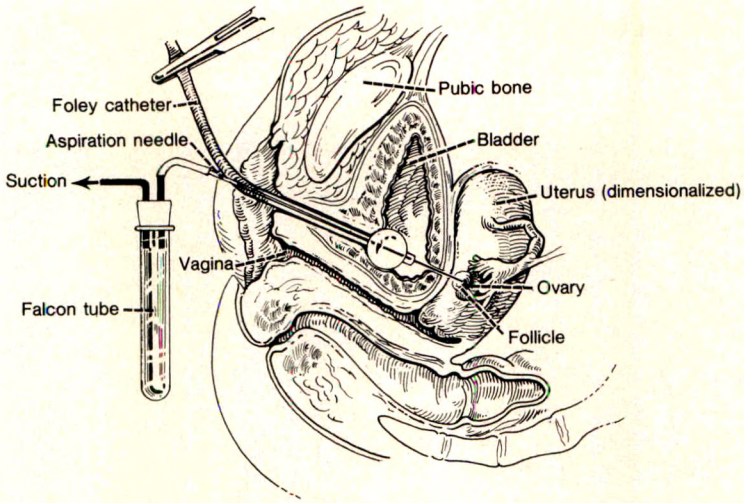


Fig. 1. Diagram of the transurethral follicular aspiration system.

be used as long as the pregnancy rate will not be affected.

In 1981 Lenz et al.[1] were the first to report successful oocyte recovery by using ultrasound-guided follicular aspiration. Since then several techniques using transabdominal,[1-4] transvaginal,[5, 6] and transurethral[7] methods with or without transducer-attached needle guides have been described with variable success. Ultrasound guidance allows oocyte retrieval in patients with inaccessible ovaries that would not otherwise be candidates for laparoscopic retrieval.

In this article we report on our experience at the Medical College of Georgia with ultrasound-guided transurethral follicular aspiration for in vitro fertilization and compare these results with those of laparoscopic retrievals done by the same team of physicians with identical follicular recruitment protocols and embryo laboratory techniques.

Material and methods

Thirty-nine follicular aspirations for in vitro fertilization were performed in 30 patients at the Medical College of Georgia from July 1985 through July 1986. All cycles performed during this time were included in the study. Group 1 consisted of 21 cycles in 16 patients in whom oocyte retrievals were performed by three-puncture laparoscopy. Group 2 consisted of 18 oocyte retrievals performed in 16 patients in whom ultrasound-guided transurethral follicular aspiration was used. Two patients who underwent laparoscopic aspiration on the first cycle and then selected ultrasound-guided aspiration on a subsequent cycle are included in the data. The protocol for the ultrasound-guided aspiration was approved by the Human Assurance Committee of the Medical College of Georgia. All patients were given the option of ultrasound aspiration regardless of their ovarian accessibility, and informed consent was obtained. The indications for in vitro fertilization in group 1 were tubal factor (n = 13), idiopathic infertility (n = 4), male factor (n = 3), and cervical factor (n = 1). The indications in group 2 were tubal factor (n = 17) and cervical factor (n = 1).

Follicular recruitment consisted of 100 mg clomiphene citrate (Serophene; Serono Laboratories Inc., Randolph, Massachusetts) and 150 IU human menopausal gonadotropin (Pergonal; Serono) for 5 consecutive days, starting on cycle day 3 or 5. Follicular monitoring by daily ultrasonographic and estradiol measurements was started on the sixth day of treatment and subsequent human menopausal gonadotropin injections were individualized based on these parameters. When a dominant follicle ≥18 mm and two secondary follicles ≥14 mm were observed, together with a rising estradiol response, 5000 IU human chorionic gonadotropin (hCG) was given and oocyte retrieval scheduled 35 hours later. Serial blood samples drawn 1 or 2 days before the day of hCG administration and at 9 AM, 12 noon, 4 PM, and 10 PM on the day of hCG administration were assayed for both luteinizing hormone and progesterone to detect a spontaneous luteinizing hormone surge.

Oocyte retrievals were performed as outpatient procedures under general or regional anesthesia. The laparoscopic aspirations were done with 100% CO_2 pneumoperitoneum, a second puncture for an atraumatic grasping forceps (R. Wolf Medical Instruments Corp., Rosemont, Illinois) and a third puncture for the aspirating needle.

The transurethral ultrasound-guided aspirations were performed by inserting the aspirating needle through a side slit made on a 20 French Foley catheter after cutting 0.5 cm from the tip of the catheter

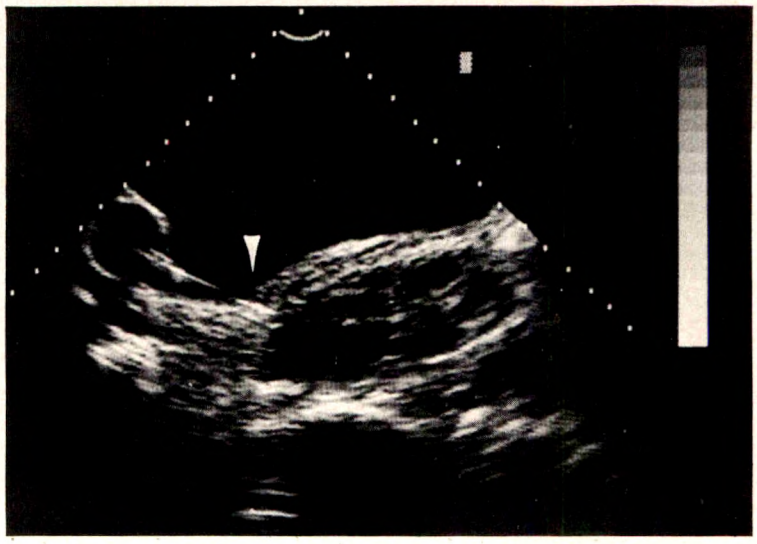


Fig. 2. Longitudinal real-time sector scan. The aspirating needle tip can be seen in the bladder wall *(arrow)* before entering a preovulatory follicle.

(Fig. 1). The catheter was used to empty, rinse, and fill the bladder with warm Ringer's lactate. Simultaneous ultrasound guidance was performed transabdominally with an ATL 4000 sector scanner and a 3.0 MHz transducer. Once the follicle to be punctured was identified the tip of the aspirating needle was guided to the bladder wall adjacent to the follicle. The position of the tip of the needle was verified by transverse and longitudinal scanning, and then the needle was advanced into the follicle (Fig. 2). The needle used in both groups was a 22 cm 14-gauge single-lumen Teflon-lined needle with an internal diameter of 1 mm (Rocket USA, Branford, Connecticut) attached to a 10 ml sterile tissue culture tube (Falcon No. 3033). Aspiration was done by continuous wall suction at 100 to 110 mm Hg with a thumb control. If the oocyte was not found in the original aspirate, the follicle was flushed with an equal volume of sterile culture media containing 100 IU/ml sodium heparin.

The oocytes collected were classified as mature or immature based on morphologic appearance of the cumulus and corona cells. Mature oocytes were incubated for 6 to 7 hours and immature oocytes for 24 to 30 hours in Ham's F10 with 7.5% heat-inactivated serum before insemination with 50,000 to 500,000 washed motile sperm. Oocytes were examined for evidence of fertilization 14 to 18 hours after insemination by removing cumulus and corona cells with a glass micropipette. Fertilized oocytes were transferred to Ham's F10 containing 15% serum. Unfertilized oocytes were transferred to fresh insemination medium and reinseminated with 50,000 to 500,000 washed motile sperm obtained from a second fresh semen sample from the husband. Cleaving embryos were transferred 55 to 80 hours after oocyte retrieval. Triploid embryos were not transferred. The luteal phase was supported with 12.5 mg progesterone in oil administered intramuscularly for 4 days followed by 10 days of 25 mg progesterone vaginal suppositories administered twice daily.

A serum β-subunit human chorionic gonadotropin (β-hCG) titer was drawn 13 to 15 days after transfer, and if positive it was repeated weekly until a gestational sac and fetal pole could be identified. Real-time ultrasonography was scheduled 4 to 5 weeks after transfer. A clinical pregnancy was diagnosed as two elevated serum β-hCG titers with histologic or ultrasonographic evidence of pregnancy. Statistical evaluation was performed by χ^2 analysis.

Results

Oocyte recovery rates for laparoscopic retrievals (group 1) and ultrasound-guided oocyte retrievals (group 2) are shown in Table I. The oocyte recovery rate was not significantly different between the groups. In group 1 a mean of eight follicles were aspirated per procedure, which is higher than a mean of six follicles per procedure for group 2. Consequently, in group 1 a higher mean number of oocytes was collected per procedure than in group 2 (seven versus four, respectively). None of these differences were statistically significant.

Table II illustrates the fertilization, cleavage, and pregnancy rates for the study groups. Fertilization and cleavage rates were slightly better for the ultrasound group but the difference did not reach statistical sig-

Table I. Comparison of oocyte recovery rate between laparoscopic and ultrasound-guided oocyte retrieval for in vitro fertilization

	Group 1 (laparoscopy)	*Group 2 (ultrasound)*
No. of retrievals	21	18
No. of follicles aspirated	177	103
No. of oocytes collected	150	77
Recovery rate (%)*	85	75

*No significant differences.

Table II. Fertilization, cleavage, and pregnancy rates of laparoscopic and ultrasound-guided oocyte retrieval for in vitro fertilization

	Laparoscopy	*Ultrasound*
No. of retrievals	21	18
No. of ooctyes collected	150	77
No. of fertilized oocytes*	104 (69%)	61 (79%)
No. of triploid*	12	2
No. of embryos transferred*	85 (82%)	55 (90%)
No. of clinical pregnancies	5	3
Rate per transfer (%)*	25	18
Rate per retrieval (%)*	24	17

*No significant differences.

nificance. The clinical pregnancy rate of the laparoscopy group (25% per transfer and 24% per retrieval) was not statistically different from the clinical pregnancy rate of the ultrasound group (18% per transfer and 17% per retrieval).

All laparoscopies and ultrasound-guided oocyte retrievals were well tolerated by the patients. Surgical complications in the laparoscopy group included a wound hematoma that resolved with conservative management and one urinary tract infection. One patient in the ultrasound group developed a urinary tract infection.

Postoperative hematuria in the ultrasound group was estimated to be <50 ml per patient and cleared within 6 hours of the procedure in all patients.

Comment

The results presented above indicate that oocyte aspiration by the transurethral ultrasound-guided method compares favorably with laparoscopic retrieval. We found no significant differences between the two methods with regard to oocyte retrieval, fertilization, cleavage, or pregnancy rates. These initial results are encouraging, although further experience with the transurethral-guided method will be required to draw definitive conclusions as to whether pregnancy rates are enhanced or compromised by this method.

When in vitro fertilization was first used in a clinical setting, ultrasound technology had not developed to its current level of capability and resolution; thus laparoscopy was the only viable option available for oocyte retrieval. Before the availability of ultrasound-guided methods, several surgical procedures were often required before the in vitro fertilization cycle so that the ovaries were made accessible for follicular aspiration.[8, 9] The advent of modern ultrasound technology has enabled in vitro fertilization centers worldwide to use ultrasound-guided aspiration as the method of choice for oocyte retrieval[1-7] and has eliminated the need in most cases for surgical intervention to maintain ovarian access. In fact, ultrasound-guided aspiration is actually made easier in cases in which the ovary is fixed by adhesions because the ovary is not able to move away from the aspirating needle. We now routinely perform transurethral-guided ultrasound aspiration in approximately 80% of our patients who undergo in vitro fertilization and have yet to encounter a patient in who we fail to retrieve at least one oocyte. The use of ultrasonography avoids exposure of the oocyte to the high concentration of CO_2 that occurs during laparoscopy when pneumoperitoneum is established with 100% CO_2. Exposure to CO_2 has been associated with poor in vitro embryo development in rabbits.[10]

The transurethral approach has several advantages, as well as disadvantages, over other ultrasound-guided techniques. Transabdominal puncture, used by several groups,[1-4] has the disadvantage that the aspirating needle passes through the abdominal wall, as well as the anterior and posterior bladder walls. In addition, several punctures through the abdominal wall are generally required. Another approach favored in several in vitro fertilization centers is the transvaginal method, wherein the aspirating needle is passed through the vaginal wall and into the ovary.[5-6] Although this technique has been used with success, it may require the use of specially modified vaginal ultrasound transducers and needle guides that are quite expensive. This method also requires puncture through an unsterile vaginal mucosa that can be well vascularized under the influence of ovarian steroids, both conditions that might increase the morbidity associated with the procedure. In our experience the transurethral method is associated with very low morbidity. In addition, if the ovaries are located high in the pelvis the bladder may still have to be entered from the vagina to reach the follicles when a transvaginal approach is used.[6] An advantage of the transvaginal method is that it is more practical than the transurethral route when the ovaries are located either behind the uterus or in the cul-de-sac. Further studies to compare the transurethral, transvaginal, and transabdominal aspiration tech-

niques will be necessary to determine which one is the best for in vitro fertilization.

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Dr. Jeffrey Kerotkin, Atlanta, Georgia. Did you have any trouble stabilizing the ovary under ultrasound guidance? Was there any difficulty with the ovary moving out of the way when you tried to puncture it to retrieve the eggs?

Dr. Padilla. Yes, we did. We used two maneuvers to stabilize the ovary. One of them was to attach a tenaculum to the cervix, pull the cervix the way you pull a utero-ovarian ligament, and stabilize it. The other maneuver was transabdominal pressure. You push the ovary down and pull it from down below and you can stabilize it and reach it.

Dr. Robert Bowden, Lynchburg, Virginia. This method was described by Stuart Campbell, and there are multiple advantages of not requiring an operating room facility, which is certainly cost-effective, and not having the problems of general anesthetic, etc. He also stated that as they got into the technique their retrieval rate became equal to the retrieval rate with laparoscopy and that also they did find that the amount of fluid that was put into the bladder was critical as far as postoperative hematuria and the possibility of a paravesical hematoma. Would you comment on that aspect.

Dr. Padilla. I think that you have to look at the amount of fluid. We pretty much used the amount of fluid that was necessary to visualize the ovaries. We have seen the other data and they showed, like we show, that there is no difference in terms of recovery rate. In terms of the volume of fluid used, it varies from patient to patient. Sometimes you can see with 300 ml and sometimes you require 400 or 450 ml to visualize. We have not seen any difference in terms of the incidence of hematuria, and our incidence has been minimal using a 14-gauge needle.

Unknown speaker. Dr. Padilla, you have mentioned that one of the purposes of this was to make the technology less invasive and less expensive if you use general or spinal anesthetic. Is it really cheaper or less invasive?

Dr. Padilla. The only thing that was cheaper was the professional charge. We did offer a local anesthetic to the patients but they all wanted at least an epidural; one patient desired a saddle block and she tolerated it pretty well. She felt the ovarian punctures but she did not feel the bladder manipulation. We did discount the professional fee but it takes about as much time to do the ultrasound aspiration as to do the laparoscopy; probably ultrasonography takes a little longer than laparoscopy.

TRANSACTIONS OF THE SEVENTH ANNUAL MEETING OF THE SOCIETY OF PERINATAL OBSTETRICIANS

The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores

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The relationships among the fetal biophysical profile, umbilical cord blood pH, and Apgar scores were investigated in 124 patients undergoing cesarean section before the onset of labor. Based on a standard for the diagnosis of fetal acidosis of an umbilical cord arterial pH <7.20, the sensitivity, specificity, and positive and negative predictive values of the fetal biophysical profile score were 90%, 96%, 82%, and 98%, respectively. When the combination of a nonreactive nonstress test and absent fetal breathing was used as the "abnormal test," the sensitivity, specificity, and positive and negative predictive values were 100%, 92%, 71%, and 100%, respectively. The efficacy of the fetal biophysical profile to indicate fetal acidosis was found to be superior to the 1- and 5-minute Apgar scores in sensitivity and positive predictive value. These data suggest that the biophysical profile is very accurate in the identification of the fetus with acidemia. The first manifestations of fetal acidosis are nonreactive nonstress testing and loss of fetal breathing; in advanced acidemia fetal movements and fetal tone are compromised. A new protocol of antepartum fetal evaluation is suggested based on individual biophysical components rather than the score alone. (Am J Obstet Gynecol 1987;157:627-31.)

Key words: Fetal biophysical profile, umbilical cord pH, Apgar score, fetal acidosis, cesarean section

The fetal biophysical profile (nonstress test, fetal breathing movements, fetal movements, fetal tone, amniotic fluid volume, and placental grading) has been shown to be more accurate in the identification of the compromised fetus than any other single test during the antepartum period.[1-7] The most important factor in the sensitivity of this testing method is the combination of acute (nonstress test, fetal breathing movements, fetal movements, fetal tone) and chronic (amniotic fluid volume) markers of the fetal condition. All previous studies have attempted to correlate the fetal biophysical profile with parameters of perinatal outcome after either spontaneous or induced labor.[1-8] The inherent drawbacks of these studies have included the long interval between the performance of the biophysical assessment and the birth of the infant, with the corresponding effects of labor and drugs on the fetus. To minimize these problems, we examined the relationship between the fetal biophysical profile and perinatal outcome, as determined by umbilical cord blood pH and Apgar scores, in patients undergoing cesarean section before the onset of labor.

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Material and methods

This prospective study included 124 consecutive patients with singleton pregnancies and gestational ages between 26 and 43 weeks who were delivered by cesarean section. The patients had intact membranes and were not in labor. Infants with congenital anomalies were excluded. In all cases a fetal biophysical profile

Table I. Efficacy of the fetal biophysical variables to predict fetal acidosis

Biophysical variable	*Definition of abnormal test*	*Sensitivity*		*Specificity*	
		%	*n*	*%*	*n*
Fetal biophysical profile score	≤7	90	18/20	96	100/104
Nonstress test, fetal breathing movements	Nonreactive nonstress test and no breathing	100	20/20	92	96/104
Nonstress test	≤1 acceleration in 20 min	100	20/20	76	79/104
Fetal breathing movements	<30 sec	100	20/20	64	67/104
Fetal movements	<3	50	10/20	96	100/104
Fetal tone	Compromised or absent	45	9/20	100	104/104
Amniotic fluid	<1 cm	35	7/20	93	97/104
Amniotic fluid	<2 cm	45	9/20	86	89/104
Placental grading	Grade III	5	1/20	94	98/104

n = Number of fetuses.

Table II. Comparison of the efficacy to predict fetal acidosis of the fetal biophysical profile and Apgar scores

Abnormal test	*Sensitivity* (%)*	*Specificity (%)*	*Positive† predictive value (%)*	*Negative predictive value (%)*
1. Nonreactive nonstress test and breathing absent	100	92	71	100
2. Biophysical score ≤7	90	96	82	98
3. 1 min Apgar score <7	65	75	33	92
4. 5 min Apgar score <7	40	93	53	89

*1 versus 3, $p < 0.005$; 1 versus 4, $p < 0.001$; 2 versus 4, $p < 0.005$.

†1 versus 3, $p < 0.005$; 2 versus 3, $p < 0.001$.

was determined within 6 hours before the cesarean section. Most of the patients (95 of 124; 77%) were transferred to the University of Connecticut Health Center because of pregnancy complications. The primary indications for delivery/cesarean section were: severe pregnancy-induced hypertension (55 patients; 45%), elective repeat cesarean section (29 patients; 23%), intrauterine growth retardation with breech presentation (15 patients; 12%), placenta previa (14 patients; 11%), fetal macrosomia (four patients; 3%), and other (seven patients; 6%). Of the 124 cesarean sections, 70 (56%) were performed under epidural anesthesia and the remaining 54 (44%) were performed under general anesthesia.

The fetal biophysical profile was determined according to a method previously described. Each biophysical variable was scored as 2, 1, or 0 according to the criteria cited in a previous study.[5] The maximal score was twelve and the minimal score was 0. Immediately after delivery of the infant, a segment of the umbilical cord was doubly clamped and blood was separately collected from both artery and vein into plastic heparinized syringes. Blood samples were then transported in ice for acid-base analysis, which was done by a means of an automatic pH/blood gas analyzer (Corning Medical Model 178). All pH measurements were performed within 5 minutes after the blood collection. Apgar scores were measured at 1 and 5 minutes by a neonatal fellow or attending physician who used a stopwatch. For the purpose of this study, fetal acidosis was defined as umbilical cord arterial pH <7.20.

Statistical analysis was by χ^2 or Fisher's exact test when appropriate (for categoric variables) and Student's *t* test (for continuous variables).

Results

A total of 124 patients were included in the study. Mean gestational age was 34 weeks (range 26 to 43 weeks). The fetal biophysical profile score was ≥8 in 102 patients (82%) and ≤7 in the remaining 22 patients (18%). A total of 20 fetuses with acidemia were encountered (cord arterial pH <7.20). The efficacy of the biophysical scoring as well as of the individual biophysical components to predict fetal acidosis is shown in Table I. In the 102 fetuses with biophysical scores of ≥8 the mean (±SD) cord arterial pH was 7.28 (±0.04) (range 7.10 to 7.38). In this group two fetuses (2%) were acidotic, both of which had scores of 8 with nonreactive nonstress test and absent fetal breathing. Thirteen fetuses with scores between 5 and 7 had a mean (±SD) cord arterial pH of 7.19 (±0.06) (range 7.19 to 7.40), and nine fetuses (69%) were acidotic. Nine fetuses with scores of 4 had a mean (±SD) cord arterial pH of 6.99 (±0.10) (range 6.85 to 7.19), and all (100%)

Positive predictive value		*Negative predictive value*	
%	*n*	%	*n*
82	18/22	98	100/102
71	20/28	100	96/96
44	20/45	100	79/79
35	20/57	100	67/67
71	10/14	91	100/110
100	9/9	90	104/115
50	7/14	88	97/110
38	9/24	89	89/100
14	1/7	84	98/117

were acidotic. The differences in the cord arterial pH values of these three groups were statistically significant (7.28 versus 7.19, $p < 0.005$; versus 6.99, $p < 0.001$).

The relationship between the fetal biophysical activities (acute markers of the fetal condition) and cord arterial pH was also investigated. For the purpose of this analysis four groups were identified. Group 1 included 96 fetuses with reactive nonstress test and/or fetal breathing present (lasting more than 30 seconds). This group was further subdivided in 50 fetuses who had both reactive nonstress test and breathing present (mean cord arterial pH 7.28; range 7.20 to 7.38), 29 fetuses with reactive nonstress test and fetal breathing absent (mean cord arterial pH 7.28; range 7.20 to 7.38), and 17 fetuses with nonreactive nonstress test and fetal breathing present (mean cord arterial pH 7.28; range 7.20 to 7.38). Group 2 (17 fetuses) had nonreactive nonstress test and fetal breathing absent with normal movements and tone; the mean cord pH of this group was 7.20 (range 7.10 to 7.40). Group 3 (four fetuses) had nonreactive nonstress test and absent fetal breathing with compromised (present, but not normal according to the criteria[5]) movements and/or tone; mean cord arterial pH of this group was 7.16 (range 7.11 to 7.20). Group 4 (seven fetuses) had all biophysical activities absent and the mean cord arterial pH was 6.95 (range 6.85 to 7.02). These cord arterial pH differences between the four groups were statistically significant ($p < 0.005$). The frequency of fetal acidosis in groups 1, 2, 3, and 4 was 0%, 59% (10 of 17), 75% (three of four), and 100% (seven of seven), respectively. The efficacy to predict fetal acidosis (cord arterial pH <7.20) according to the combination of reactive nonstress test and/or fetal breathing present as the normal test, and nonreactive nonstress test and fetal breathing absent as the abnormal test, was found to be 100%, 92%, 71%, and 100%, respectively (Table I).

Of the individual biophysical components (Table I), the nonstress test and fetal breathing had the highest sensitivities and negative predictive values (both 100%); their combination (with nonreactive nonstress test and fetal breathing absent as the abnormal test) increased the specificity and positive predictive value to 92% and 71%, respectively. Fetal tone had the highest positive predictive value (100%) but low sensitivity (45%).

When the fetal biophysical profiles of the 20 fetuses with acidemia were analyzed, the following were observed. All 20 fetuses had nonreactive nonstress test and absent fetal breathing. When movements and tone were normal (10 fetuses), the mean (±SD) cord arterial pH was 7.16 (±0.03); among these 10 fetuses there was only one death, which was attributed to prematurity. Nine survived with no sequelae. When the movements and tone were partially compromised (three fetuses), the mean (±SD) cord arterial pH was 7.15 (±0.04) and none died. When movements and tone were absent (seven fetuses), the mean (±SD) cord arterial pH was 6.95 (±0.06); four of these seven fetuses died, two survived with major hypoxic sequelae, and one survived with no sequelae. The mean cord arterial pH was significantly lower when all fetal biophysical activities were absent (6.95 versus 7.15 or 7.16, $p < 0.001$). Of the 20 fetuses with acidemia, seven (35%) had amniotic fluid pockets <1 cm and nine (45%) had pockets <2 cm in vertical diameter. Of the 104 nonacidotic fetuses, seven (7%) had amniotic fluid pockets <1 cm and 15 (14%) had pockets <2 cm in vertical diameter. These differences were statistically significant (35% versus 7%, $p < 0.005$; and 45% versus 14%, $p < 0.01$).

There were 39 fetuses with a 1-minute Apgar score <7 and only 13 were acidotic; of the 85 fetuses with a 1-minute Apgar score ≥7, seven were acidotic. There were 15 fetuses with a 5-minute Apgar score <7 and only eight were acidotic; of the 109 fetuses with a 5-minute Apgar score ≥7, 12 were acidotic. Table II compares the efficacy of the fetal biophysical profile score (≤7), nonreactive nonstress test and fetal breathing absent, and Apgar scores (≤7) at 1 and 5 minutes in predicting the fetus with acidemia. The sensitivity of the combination of nonreactive nonstress test and breathing absent was significantly better than the 1- and 5-minute Apgar scores ($p < 0.005$ and $p < 0.001$, respectively), and the sensitivity of the biophysical score was better than the 5-minute Apgar score ($p < 0.005$). In terms of positive predictive value, the combination of nonreactive nonstress test and breathing absent was significantly better than the 1-minute Apgar score ($p < 0.005$) and the biophysical score was better than the 1-minute Apgar score ($p < 0.001$). There were no differences in specificity and negative predictive value.

Comment

The present data suggest that there is a significant relationship between the fetal biophysical profile and fetal acid-base status at the time of testing. The sensi-

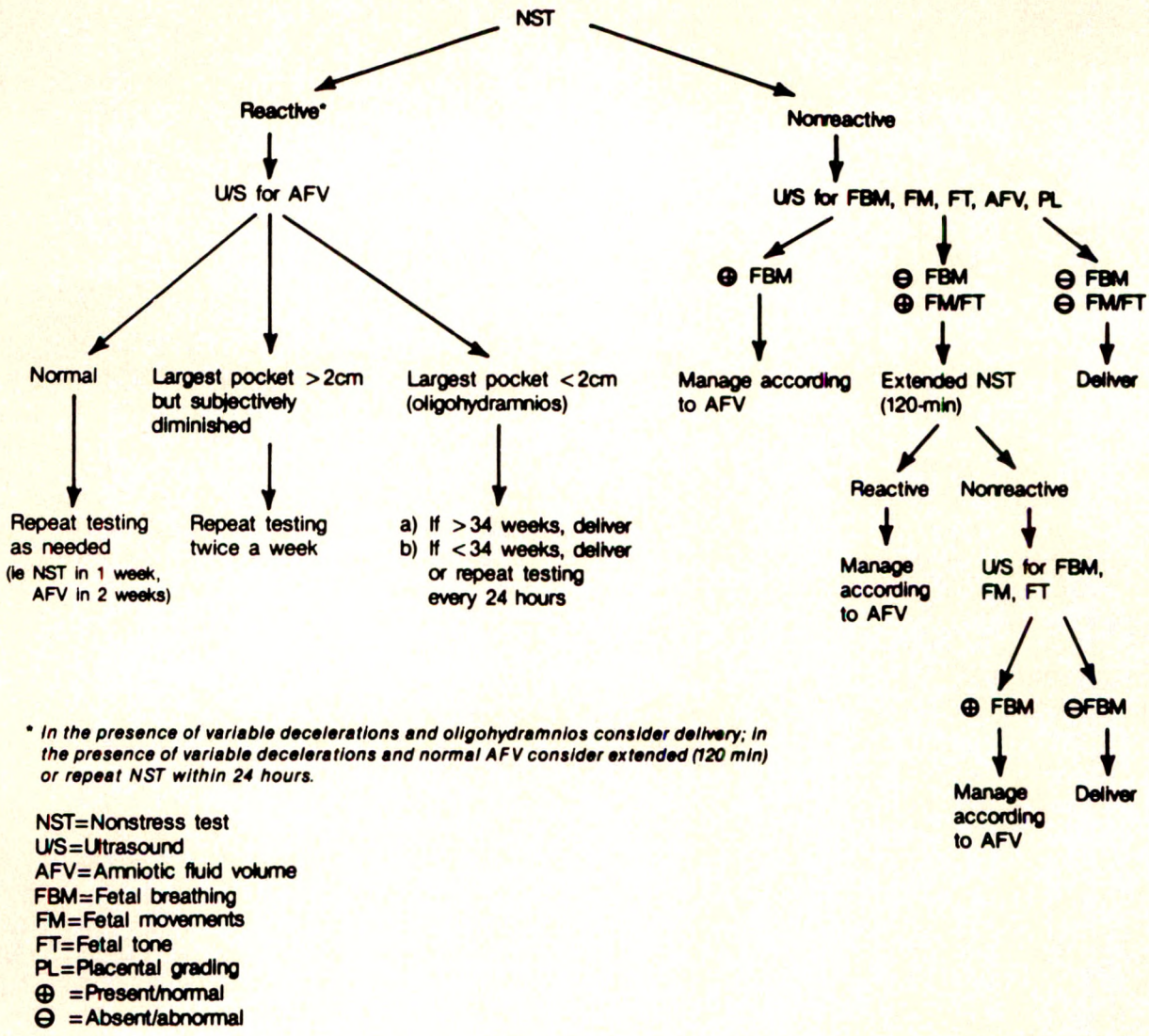


Fig. 1. Protocol of antepartum fetal evaluation with intact membranes.

tivity of this testing method was 100% if the individual fetal biophysical components were considered rather than the score.

The first manifestations of fetal acidosis are nonreactive nonstress testing and loss of fetal breathing; in advanced acidemia fetal movements and tone are compromised. The acute markers of the fetal condition were also found to correlate with the long-term outcome of the 20 fetuses with acidemia. When all biophysical activities were absent (seven fetuses) the outcomes included four deaths, two survivors with major neurological sequelae, and one normal survivor. The above concept of different levels of sensitivity to hypoxia and acidosis of the fetal central nervous system centers, which control the biophysical activities,[9] is very important in the correct interpretation and application of the information obtained from the biophysical monitoring of the fetus. Indeed, the sensitivity in predicting the acidotic fetus was 100% when the combination of nonreactive nonstress test and breathing absent was used as the abnormal test.

Amniotic fluid volume, unlike the other biophysical variables, is not acutely influenced by alterations in fetal central nervous system function and is a chronic marker of the fetal condition. It is included in the fetal biophysical profile because of the high correlation between decreased amniotic fluid volume and increased incidence of abnormal pregnancy outcome.[1-5, 10] The present data support an increased frequency of oligohydramnios in the presence of fetal acidosis. Fetuses with decreased amniotic fluid volume are at a high risk for cord compression and in utero death regardless of the presence or absence of the other biophysical variables. The presence of a Grade III placenta was not found

to predict fetal acidosis; nonetheless, in our institution placental grading is still included in the fetal biophysical profile because Grade III placentas have been found to be associated with increased incidence of abnormal fetal heart rate patterns (44.4%) and abruptio placentae (14.8%) during labor.[5] The value of including placental grading is to alert the physician during the intrapartum period to the presence of a Grade III placenta.

Considering the physiology and pathophysiology of fetal biophysical activities, and because the sensitivity for predicting the fetus with acidemia is best determined by the combination of nonreactive nonstress test and fetal breathing absent as the abnormal test, we recommend an alternative scheme of antepartum fetal evaluation based on the individual biophysical components rather than the score (Fig. 1). This scheme includes assessment of both an acute (nonstress test and/or fetal breathing) and a chronic (amniotic fluid volume) marker. We have applied this protocol of antepartum fetal evaluation to 6543 fetuses over a 4-year period, and we have never encountered a fetal death of a structurally normal fetus within a week after a reassuring biophysical assessment.[11]

This alternative protocol of antepartum fetal evaluation, without including the scoring of the biophysical variables, will increase the sensitivity of this testing method and eliminate the problems encountered in the clinical interpretation and application of the obtained information. Our present data also suggest that the fetal biophysical profile is a better predictor of the acidotic fetus than the Apgar scores. This should not be surprising because the fetal biophysical profile was designed to predict fetal asphyxia, while Apgar scores were designed to assess the need and extent of neonatal resuscitation. The understanding of the physiology and pathophysiology of the fetal biophysical activities, as well as the significance of oligohydramnios, is imperative for the proper clinical application of this tool and therefore for the improvement of the quality of perinatal care.

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Fetal cerebellar growth unaffected by intrauterine growth retardation: A new parameter for prenatal diagnosis

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Nineteen pregnant women with a clinical suspicion of intrauterine growth retardation and with gestational age confirmed by early ultrasound examination were referred to our departments for sonographic evaluations. Multiple biometric parameters were obtained, including the transverse cerebellar diameter by use of the electronic calipers of the machine. A prenatal diagnosis of intrauterine growth retardation was made in all cases based on: (1) the transverse cerebellar diameter being consistently correlated with gestational age as predicted by the last menstrual period, whereas most of the other measurements were consistently discrepant with the transverse cerebellar diameter by more than 2.5 weeks (i.e., more than 2 SD above the mean), and (2) the estimated fetal weight of all fetuses being equal to or less than the tenth percentile for gestational age. Neonatal examination confirmed all fetuses to be growth retarded with birth weights at or below the tenth percentile for gestational age. These findings indicate that growth of the transverse cerebellar diameter is unaffected by intrauterine growth retardation; thus this sonographic measurement may serve as an independent and reliable correlate of gestational age against which potential deviations of growth may be compared. (Am J Obstet Gynecol 1987;157:632-8.)

Key words: Cerebellum, intrauterine growth retardation, ultrasound, gestational age

Intrauterine growth retardation (IUGR) occurs in about 2% to 10% of all infants born in developed countries.[1] Despite overall advances made in perinatal medicine, the exact cause of IUGR remains unclear, and the mortality rate among these fetuses is still higher than in normal pregnancies.[2] In light of the increased death rate associated with IUGR, early detection of reduced fetal growth is essential so that intense fetal surveillance may be instituted, permitting the detection of fetal compromise and the opportunity for intervention before the fetus is in jeopardy. Current methods used to detect fetal growth retardation are hampered by the lack of precise estimate of gestational age in most cases, leading to difficulty in determining whether a fetus is truly growth retarded, constitutionally small, or appropriately grown but with an incorrect gestational age. It would be desirable, however, for some biometric parameters to be unaffected by growth aberrations, thus providing a standard against which disparate growth

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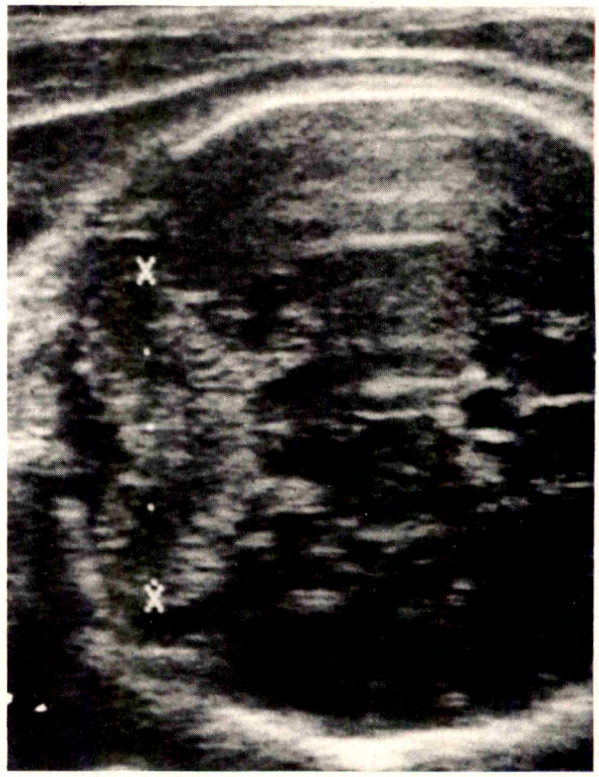


Fig. 1. Ultrasonogram of fetal head showing the thalami *(th)* and the cerebellum *(CB)*. The *dotted lines* indicate the points from which measurements were taken.

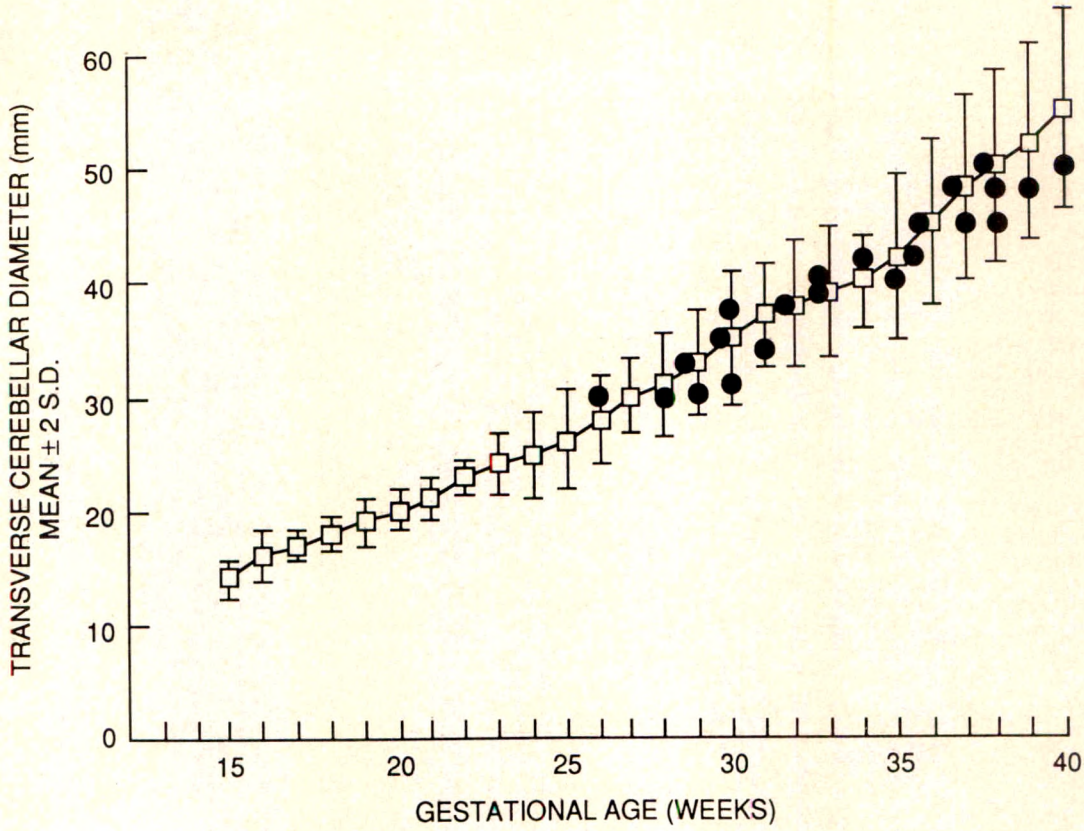


Fig. 2. The mean ±2 SD of normal transverse cerebellar diameter plotted against the gestational age according to Goldstein et al. The transverse cerebellar diameter of all growth-retarded fetuses remained within normal limits as depicted by *dots*. (Reproduced with permission from Goldstein I, Reece EA, Pilu G, Bovicelli L, Hobbins JC. Am J Obstet Gynecol 1987;156:1065-9.)

could be compared and the diagnosis of fetal growth retardation established.

The purpose of this communication is to report a study in which fetuses suspected of having growth retardation were examined sonographically to determine whether a comparison between the gestational age predicted by the transverse cerebellar diameter and that predicted by multiple other biometric parameters could be used as an adjunctive method in the prenatal diagnosis of IUGR.

Patients and methods

A prospective study was conducted in 19 pregnant women with known last menstrual periods confirmed by first-trimester or early second-trimester ultrasound examination, who were referred to the perinatal unit at Yale–New Haven Hospital or the pathophysiology unit of the University of Bologna with a clinical suspicion of fetal growth retardation. These fetuses were subjected to sonographic examinations, and the measurements were obtained by use of the electronic calipers of the ultrasound machines (General Electric RT 2600 and RT 3000; GE Corp., Rancho Cordova, California). In each patient several biometric measurements were obtained, including the biparietal diameter, the occipitofrontal diameter, the calculated head circumference, cephalic index, abdominal circumference, estimated fetal weight, long bone measurements, and the transverse cerebellar diameter. The latter measurement was obtained from the outer-to-outer margins of the cerebellum, after its identification in the posterior fossa (Fig. 1). Some patients received serial examinations at approximately 2- to 3-week intervals. Cerebellar growth in normal pregnancies has been previously studied, and nomograms have been generated.[3, 4] In our study[4] we found a high degree of correlation ($p < 0.0001$) between transverse cerebellar diameter and gestational age, with the overall 2 SD being ±2.4 weeks. Before 24 weeks the transverse cerebellar diameter in millimeters was equivalent to the gestational age in weeks. However, slight flattening of the growth curve was seen beyond this point. The growth pattern was best described by a third-order polynominal equation. A determination of IUGR was

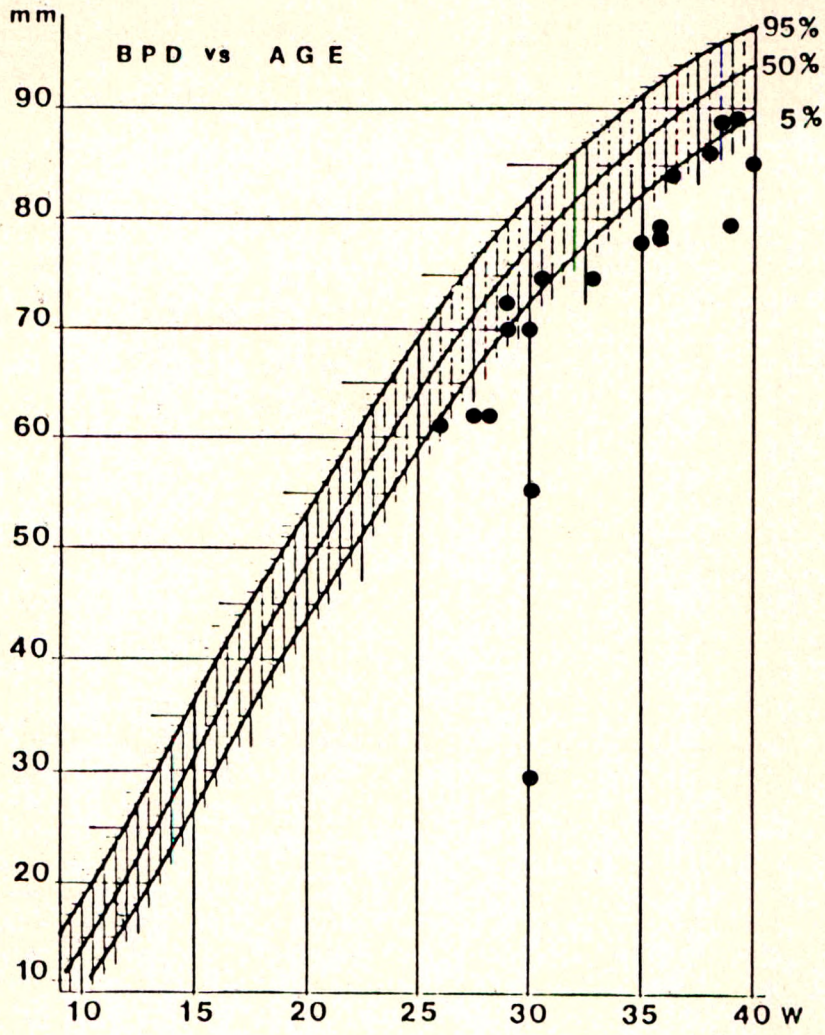


Fig. 3. Biparietal diameters *(BPD)* of normal fetuses by Jeanty and Romero. Scatter points indicate the biparietal diameter of patients in this study fell at or below the fifth percentile. (Reproduced with permission from Jeanty P, Romero R. Obstetrical ultrasound. New York: McGraw-Hill, 1984.)

made by use of this nomogram if the estimated fetal weight was at or below the tenth percentile for the gestational age predicted by the transverse cerebellar diameter, and if a discrepancy between the gestational age by the transverse cerebellar diameter and the other measurements existed and was greater than 2.5 weeks. Neonatal information collected at birth included the birth weight, length, ponderal index, and a determination as to whether the fetus was growth retarded based on the percentile rank, as measured by the scale of Usher and McClean[5] or by the Lubchenco nomogram.[6]

Results

Pregnant women referred to our departments because of a suspicion of IUGR underwent diagnostic ultrasonography. The gestational ages of these pregnancies ranged from 24 to 40 weeks. In all 19 consecutive patients in whom the diagnosis of IUGR was made, the transverse cerebellar diameter correlated with the gestational age as predicted by the last menstrual period, and it remained within the mean for the gestational age (Fig. 2). The remaining biometric parameters predicted a fetus of a much earlier gestational age. The disparity in gestational age (between that predicted by transverse cerebellar diameter and that predicted by the remaining biometric parameters) was consistently greater than 2.5 weeks (i.e., greater than 2 SD above the mean). Additionally, the measurements of the biparietal diameter, the abdominal circumference, and the femur length were at the fifth percentile for gestational age (Figs. 3 to 5). Antenatal estimation of fetal weight by sonography revealed fetuses to be under the tenth percentile (Fig. 6). Neonatal evaluations revealed all fetuses to be growth retarded. Their birth weights were equal to or less than the tenth percentile for gestational age (Fig. 7).

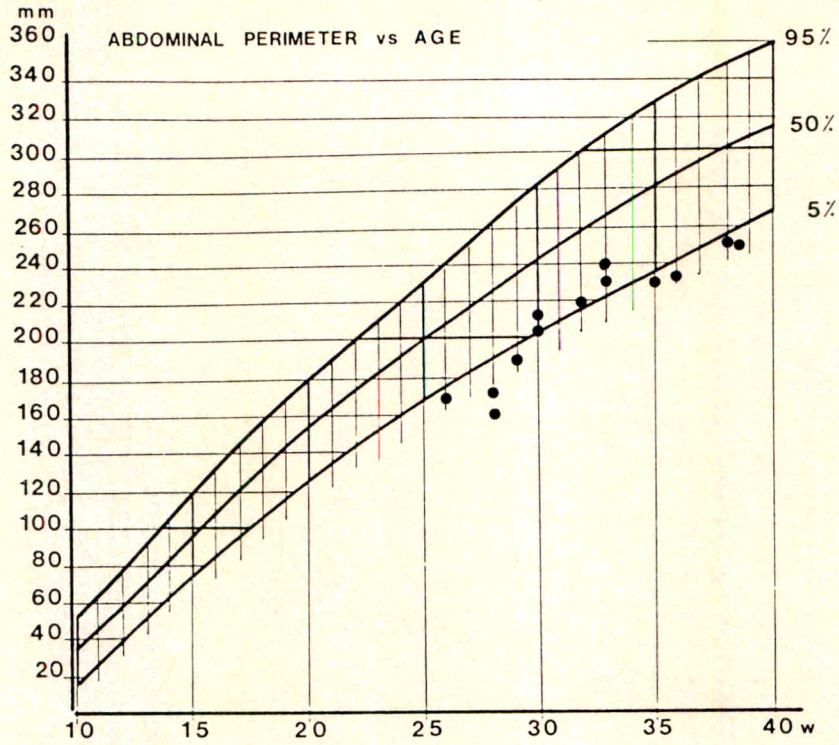


Fig. 4. Abdominal perimeter of normal fetuses by Jeanty and Romero. The scatter points indicate the abdominal circumference of patients in this study at about the fifth percentile for gestational age. (Reproduced with permission from Jeanty P, Romero R. Obstetrical ultrasound. New York: McGraw-Hill, 1984.)

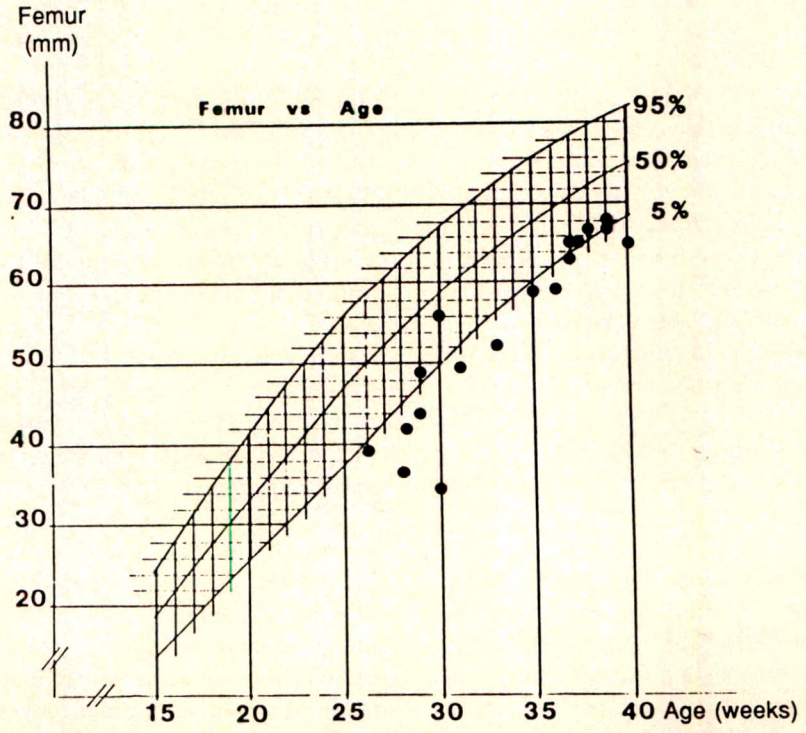


Fig. 5. Femur lengths of normal fetuses by Jeanty and Romero. Scatter points represent the femur lengths of patients in this study, which fell at or below the fifth percentile for gestational age. (Reproduced with permission from Jeanty P, Romero R. Obstetrical ultrasound. New York: McGraw-Hill, 1984.)

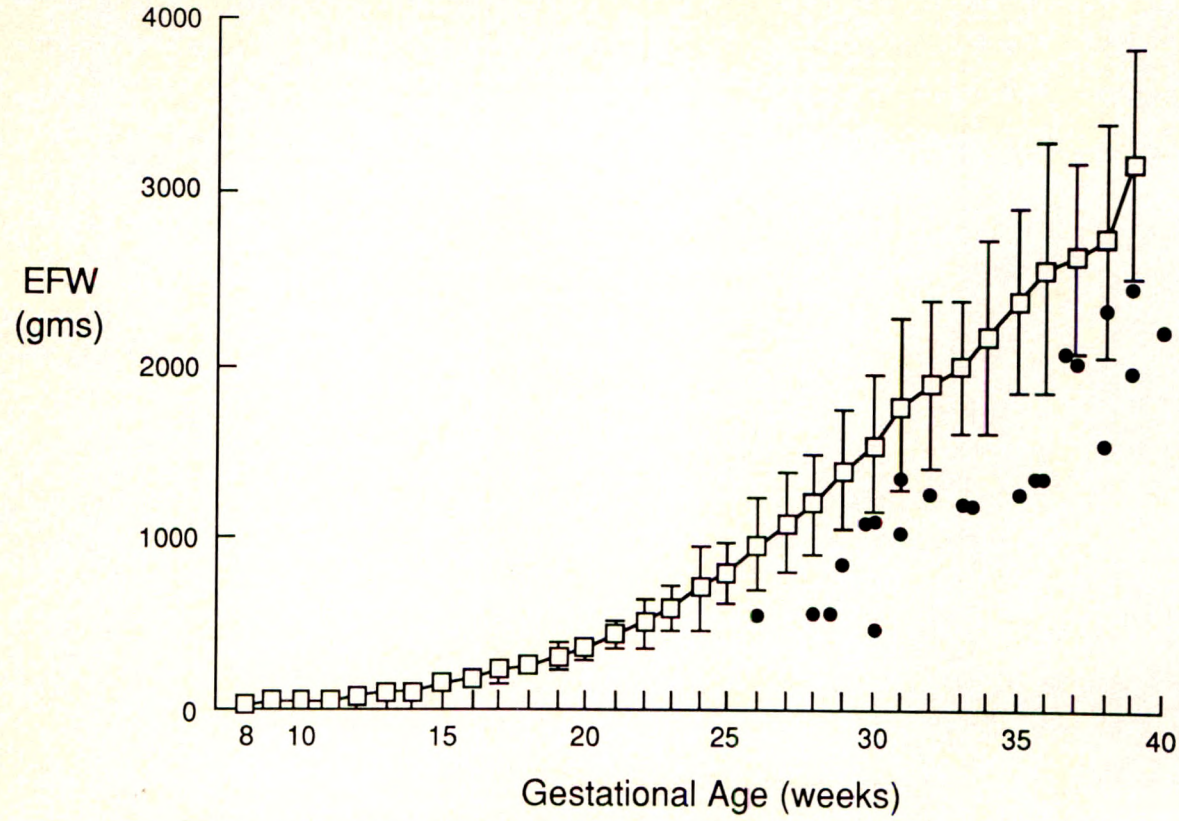


Fig. 6. Normal estimated fetal weight *(EFW)* versus gestational age by Jeanty and Romero. Scatter points represent estimated fetal weights of patients in this study, which fell below the mean. (Reproduced with permission from Jeanty P, Romero R. Obstetrical ultrasound. New York: McGraw-Hill, 1984.)

Comment

This study demonstrates that the transverse cerebellar diameter remained unaffected by fetal growth retardation, whereas most biometric parameters measured sonographically were significatly affected by the overall growth retardation process. The consistent disparity between the gestational age predicted by transverse cerebellar diameter and that predicted by most other biometric parameters was remarkable. In this relatively small series there were no false negative or false positive diagnoses.

The cerebellum is well protected in the fetal head, and it has been shown in experimental animals that this organ is the last to be affected by a decrease in blood flow.[7] Additionally, the fetus responds to decreased uteroplacental perfusion by circulatory adjustment, by which the blood supply to the brain is conserved at the expense of blood flow to the skin, muscle, and lungs.[8] Behrman et al.[7] studied the regional distribution of blood flow to term primate fetal brain in both control and asphyxiated animals. They found that the blood flow to the cerebellum was significantly higher than that to the cerebral cortex in the control group. However, in asphyxiated fetuses, although cortical blood flow decreased, blood flow to the cerebellum remained unchanged. Others have reported vasodilation of cerebellar vessels in asphyxiated fetal lambs.[9] In light of these remarkable findings one has to presume the hemodynamic adjustments observed under experimental conditions are operative in the human fetus and are important in the preservation of normal cerebellar growth, even in the presence of severe fetal growth retardation.

The inability to ascertain gestational age by ultrasound parameters in the third trimester has proved to be a major cause of undiagnosed growth-retarded fetuses. Various methods have been proposed for detecting IUGR; however, varying accuracy and detection rates have been reported with sensitivity ranging from 44% to 90%, specificity from 88% to 93%, and predictive values from 40% to 100%.[10] Some of these diagnostic methods include assessment of the biparietal diameter growth, in which there may be evidence of late flattening resulting in the biparietal diameter measuring consistently below normal. Other measurements include a formula combining the fetal abdominal circumference and the biparietal diameter to estimate the fetal weight. The rationale for this method is based on the

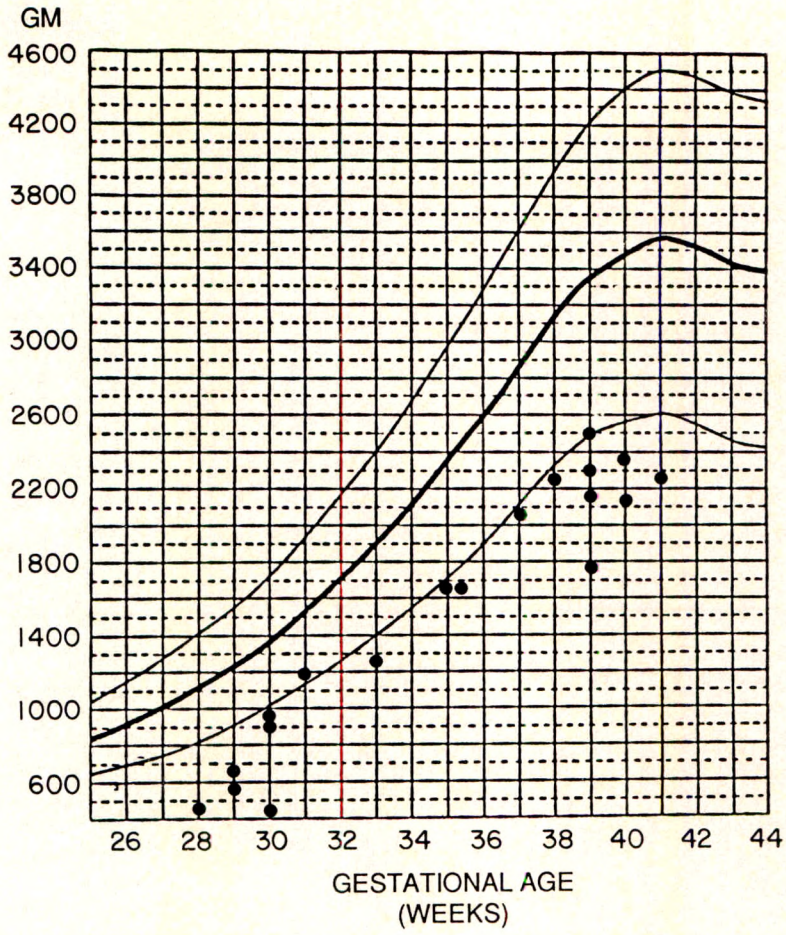


Fig. 7. Neonatal birth weight according to Usher and McClean using a sea-level population plotted against gestational age. Note the birth weights of all our patients at/or below the tenth percentile depicted. (Reproduced with permission from Usher R, McClean F. J Pediatr 1969;74:901.)

concept that the liver is the most severely affected intra-abdominal organ in decreased fetal growth and that it might lend itself to the early detection of IUGR.[11] The ratio of head circumference to abdominal circumference is another method utilized in an attempt to differentiate between the types of growth-retarded fetuses.[12] The fetal ponderal index has also been evaluated as a predictor for IUGR.[13] Other methods introduced to diagnose IUGR but found to be less sensitive include the total intrauterine volume or the 1 cm pocket of fluid.[14, 15] In spite of these various methods attempting to identify the growth-retarded fetus, a major source of frustration lies in the inability to ascertain the precise gestational age. Unfortunately, the very biometric measurements used to demonstrate abnormal growth are also the ones used to determine gestational age. This dilemma could be overcome if a parameter were available that was relatively spared by alterations in fetal growth and therefore could be used as a standard for gestational age against which all other biometric parameters could be compared. This preliminary report strongly suggests that transverse cerebellar diameter represents such a parameter.

In conclusion, measurement of the transverse cerebellar diameter can be performed on most fetuses, independent of the fetal head shape, or even when the fetus is in a direct occiput posterior position. The present lack of precise diagnostic criteria for IUGR leads to an inaccurate antenatal diagnosis. Utility of the transverse cerebellar diameter may serve as an independent and reliable indicator of gestational age and a standard against which aberrations in fetal growth may be compared.

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A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor

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In a prospective trial we enrolled 157 women at increased risk of preterm birth, randomly assigning women in a ratio of 1:2 to receive either frequent (≥5 days/wk) nursing contact, education in preterm labor symptoms, and self-palpation of uterine activity (group E, n = 50), or daily nursing contact, preterm labor education, and the Term Guard home uterine activity monitor (group EM, n = 107). Comparison of the rate of preterm birth, the incidence of preterm labor and successful tocolysis, and the mean birth weight and gestational age revealed no significant differences and suggested that beneficial effects previously attributed to monitored contraction data may in fact be the result of frequent (five or more times per week) nursing contact and careful attention to preterm labor symptoms and perceived contractions. (Am J Obstet Gynecol 1987;157:638-43.)

Key words: Term Guard uterine activity monitor, preterm birth, preterm labor

Early detection of increased uterine activity may lead to more effective tocolysis in women at risk of preterm labor. Self-palpation of uterine activity as part of an educational program for women at risk of preterm labor has been reported by Herron et al.[1] to increase the percentage of patients with preterm labor who are candidates for tocolysis, thereby reducing the rate of preterm birth. Newman et al.[2] reported, however, that self-detection of uterine contractions correlated poorly with tocodynamometer recording of uterine activity and suggested that self-detection may not be a reliable early method of detection of preterm labor. Ambulatory tocodynamometry, with the Term Guard uterine activity monitor, has been reported by Katz et al.[3-5] in a series of articles to be a safe and reliable method of uterine activity assessment that has shown promise in the early identification of women in preterm labor.

Because women using such a monitor are necessarily in daily contact with a nurse whose advice and support may lead to earlier identification of preterm labor, we

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Reprints not available.

designed a prospective random trial comparing pregnancy complications and outcomes in women at increased risk of premature delivery. One group used the Term Guard uterine activity monitor (Tokos Medical Corporation, Santa Ana, California) and a control group received instruction in self-palpation and a similar level of nursing education and support.

Material and methods

One hundred fifty-seven pregnant women at increased risk of preterm labor were recruited from area physicians (n = 136) and The Ohio State University prenatal clinic (n = 21). In all, there were 42 physicians or practice groups who contributed subjects to the study. None of the subjects had had preterm labor in the current pregnancy before enrollment, and all were between 20 and 34 weeks' gestation at entry. Informed consent was obtained, and subjects were then randomized in a ratio of 1:2 to one of two groups, E or EM, according to a protocol approved by The Ohio State University Human Subjects Review Committee. Group E patients received an initial educational session, usually lasting 60 to 75 minutes, about the symptoms and signs of preterm labor and were taught self-palpation of uterine activity. They were asked to note the presence of symptoms of preterm labor, listed in Table I, and to palpate uterine activity for 1 hour twice daily. Each group E subject was contacted by a nurse at the Columbus Term Guard Monitoring Center daily on weekdays and as needed on weekends. Women in group EM were similarly educated at entry and instructed as well in the use of the Term Guard home uterine activity monitor. They were asked to note symptoms and to record contractions for 1 hour twice daily. Group EM patients were contacted by telephone daily including weekends to transmit and interpret monitor strips and symptoms. Women in both groups were instructed to call the monitoring center if symptoms and/or increased contractions (more than four per hour) were noted and persisted for 1 hour despite lateral bed rest and oral hydration. Women with persistent symptoms and/or contractions in excess of their established baseline contraction frequency were sent to the doctor's office or to the labor and delivery suite for a cervical examination, unless the doctor specifically ordered otherwise, for example, requested instead an additional hour of home monitoring and hydration or, for those patients receiving oral tocolysis, an additional dose of medication.

Participating physicians were asked to follow broad standard guidelines of care for preterm labor, using intravenous ritodrine or magnesium sulfate or subcutaneous terbutaline in standard doses when preterm labor was diagnosed. The definition of preterm labor used in the March of Dimes Multicenter Preterm Birth Prevention Project was suggested, with parenteral tocolysis recommended for persistent uterine contractions numbering eight or more per hour if the cervix was already 2 cm dilated or 50% effaced but requiring cervical change if the cervix was <2 cm dilated and less than 50% effaced. Prophylactic tocolysis was discouraged unless the patient had a history of preterm delivery. For purposes of data analysis, preterm labor was defined as admission to the hospital and administration of either an intravenous tocolytic or multiple doses of a subcutaneous tocolytic. Once parenteral tocolysis was begun, oral tocolytics were continued in all cases until ≥35 weeks' gestation. Participating physicians were visited at least once during the study by a Tokos Medical Corporation representative and/or a project nurse who reinforced the study protocols. Medical and nursing in-service educational programs were provided to the larger referring hospitals in the area. All nurses providing daily contact and monitor interpretation were employed at the Columbus Term Guard Monitoring Center and received a standard orientation and education program provided by the Tokos Medical Corporation. A system of "primary nursing" was adopted shortly after the study started, wherein each patient in both groups had one nurse who served as her principal contact. All nurses had subjects in both groups. A nurse was available to all patients in both groups 24 hours a day, 7 days a week. The monitoring center staff was aware of the crude preterm birth rates for both groups as the study progressed.

Each birth before 37 completed weeks of gestation was reviewed by the principal investigator with the attending physician and the Term Guard nursing staff to confirm gestational age and compliance with protocol guidelines.

Table I. Symptoms of preterm labor

Uterine contractions ≥4/hr, with or without pain
Low backache
Increased vaginal discharge
Menstrual-like cramps
Pelvic pressure
Abdominal cramping
Spotting

Results

One hundred fifty-seven women had been delivered of their infants as of mid-November, 1986 (107 in group EM and 50 in group E). There were nine women (8.4%) in group EM and six women (12%) in group E who dropped out of the study. This difference is not significant. The most common reason for dropout in both groups was that the patient and/or the doctor found daily telephone contact or contraction data to be

Table II. Demographic description of study subjects

	Group E		*Group EM*	
	n	%	*n*	%
No.	44		98	
Parity (mean ± SD)	1.57 ± 1.2		1.47 ± 1.1	
Previous preterm delivery	27	61.4	60	61.2
Twins	15	34.1	25	25.2
Second-trimester loss	5	11.4	17	17.3
Cerclage	3	6.8	11	11.2
Uterine anomaly or diethylstilbestrol	3	6.8	9	9.2
Bleeding after 14 wk	1	2.3	3	3.1

Table III. Outcome of pregnancy

	Group E		*Group EM*	
	n	%	*n*	%
n	44		98	
Delivery				
<35 wk	9	20.4	23	23.5
<37 wk	23	52.3	42	42.9
Gestational age* (mean ± SD)	35.8 ± 2.7		35.7 ± 2.8	
Birth weight (mean ± SD)	2718 ± 745		2722 ± 738	

*All births ≥37 weeks were averaged as 37 weeks to avoid skew caused by postdate pregnancies.

annoying or confusing. None of these 15 women was delivered within 7 days of discontinuing the protocol, and their inclusion would not affect the results of the study. There were 98 patients in group EM and 44 in group E who continued to use the service until delivery or until 37 weeks' gestation; these 142 women constitute the population used to obtain the results.

Risk factors for preterm delivery and other demographic variables were similar in each group, as displayed in Table II. Some women had more than one risk factor. Previous preterm birth and twins were the most common reasons for enrollment in both groups. There were no subjects with triplet gestations in either group.

The incidence of prematurity, defined at 35 and 37 weeks, and the mean gestational age and birth weight are shown in Table III. Thirty-five weeks was selected for analysis because all participating physicians were aggressive in the use of parenteral tocolytics and were fully compliant with protocol guidelines through 35 completed weeks. Some did allow preterm labor after 35 weeks to progress without treatment in several instances, evenly distributed between groups E and EM. The differences in preterm birth rates are not statistically significant; analysis at 34 and 36 weeks also reveals no significant difference.

Births before 37 completed weeks were extensively reviewed and categorized, as displayed in Table IV, according to the diagnostic category that directly led to the preterm delivery: preterm labor, preterm prematurely ruptured membranes, or a medical or obstetric indication unrelated to preterm labor or preterm premature rupture of the membranes. Those preterm births that followed preterm labor were further categorized as (1) following a failed tocolytic attempt, (2) allowed by a physician because of a gestational age >35 completed weeks, or (3) occurring because of advanced cervical dilation >4 cm on arrival. None of the differences in Table IV is significant. In no case was preterm labor before 35 weeks allowed to progress without treatment unless the cervix was already >4 cm dilated on arrival.

The incidence of preterm labor was 36% in group E and 39% in group EM, as shown in Table V. The number of episodes of preterm labor eligible for treatment, the overall use of prophylactic and therapeutic tocolytics, and the gestational age at onset of preterm labor are also not statistically different.

Weeks gained from study entry until delivery or until 37 weeks averaged 7.35 ± 4.2 for the 40 patients in group E and 9.0 ± 4.4 for the 94 in group EM who did not have an indicated preterm birth. Considering only those women in each group who developed preterm labor, the number of days gained from first appearance of preterm labor until delivery or until 37 weeks' gestation was 18.6 ± 21.0 in group E and 10.9 ± 12.9 in group EM. These differences are not significant.

Table IV. Analysis of preterm births (<37 weeks' gestation)

	Group E		*Group EM*	
	n	%	*n*	%
No. delivered <37 wk	23		42	
Cause of preterm delivery				
Preterm labor	14	60.9	27	64.3
Failed tocolysis	5		10	
≥35 wk	9		15	
Cervical dilation > 4 cm	0		2	
Preterm premature rupture of membranes	5	21.7	11	26.2
Indicated	4	17.4	4	9.5

Table V. Preterm labor

	Group E		*Group EM*	
	n	%	*n*	%
n	44		98	
Subjects with preterm labor	16	36.3	38	38.8
Episodes of preterm labor	23		46	
Treated	13	56.5	28	60.9
Not treated	10		18	
35 wk	9		15	
Cervical dilation >4 cm	0		2	
Distress	1		1	
Prophylactic tocolytic used	12	27.3	26	26.5
Gestational age at first preterm labor (mean ± SD)	31.5 ± 3.8		32.1 ± 3.3	
Days gained from first preterm labor to delivery (mean ± SD)	18.6 ± 21		10.9 ± 12.9	

During the course of the study, several physicians expressed concern that participation would lead to an excessive number of unscheduled office visits for cervical examination because of the frequency and intensity of contact with subjects in both groups. We therefore looked also at the frequency of unscheduled visits, defined as an office or labor and delivery suite visit initiated by the Term Guard nurse. There were 60 unscheduled visits in 44 group E patients (1.36 ± 1.28 per subject) and 146 unscheduled visits in 98 group EM patients (1.49 ± 1.37 per subject). Seventy-three percent of group E and 74% of group EM subjects made at least one unscheduled visit.

Sample size is always an issue in clinical studies. Before beginning this study, we based estimates of sample size on rates of preterm birth seen in our preterm labor clinic population, defining risk according to the Creasy scoring system.[12] The rate of preterm birth (<37 weeks' gestation) was approximately 30%. We postulated a 30% reduction in deliveries before 37 weeks with the use of the Term Guard monitor, which would require 230 subjects in each group to achieve a one-tailed p of 0.05 and a power of 0.8. Using a 2:1 randomization schema, we planned to enroll 170 women in group E and 340 in group EM to achieve a harmonic mean of 230. However, entry criteria for this study proved to be substantially more stringent than those of the Creasy system, yielding a study population with a higher inherent risk of preterm birth. A revised power analysis at this point in the study reveals that a harmonic mean of 90 to 100 subjects (E = 75, EM = 150) will be necessary to detect a drop in the preterm birth rate from 50% to 30%. A sufficient number of undelivered subjects have been enrolled to reach that goal.

Analysis of the data reported here by the method reported by Young et al.[7] reveals that the sample size is sufficient to have detected a 50% fall in the preterm delivery rate and sufficient as well to have detected a 10-day gain from the first episode of preterm labor with a power of 0.8 and a p of 0.05.

Comment

The physiologic basis of contraction monitoring rests on a series of reports from multiple investigators correlating increased uterine contraction frequency during pregnancy and risk of preterm birth.[4, 8-10] Although normal and increased levels of uterine activity at each gestational age remain to be established, it appears from these reports that women destined to be delivered before term do, in fact, have more frequent contractile

activity throughout the latter half of pregnancy when compared with women who are delivered at term.

The next question is how best to use this association to prevent unnecessary preterm births. Main et al.[9] appear to have been successful in using the association as a screening tool to identify a subset of their population at increased risk of preterm delivery, something which had proved to be difficult with more traditional historical and risk scoring approaches in their population of indigent black women.[11]

Herron et al.[1] taught self-assessment of uterine activity to women who had been already identified by a risk score as being at high risk for preterm labor. They demonstrated a substantial drop in the preterm delivery rate, and their methods were soon widely imitated with variable results.[6, 12] The key features of preterm birth prevention programs patterned after the report by Herron et al. are identification of women at increased risk of preterm labor and intensive patient and staff education about the symptoms and signs of preterm labor. In this approach, the patient is a key participant in the early identification of preterm labor. The patient must recognize the signs and/or symptoms of preterm labor and initiate contact with the health care system. The report of Newman et al.[2] that high-risk women identified only 15% of their recorded contractions cast doubt on the reliability of a patient-based "alarm system" for the early identification of preterm labor. The development of technology capable of reliably recording and transmitting uterine activity data in a timely fashion seemed a good way to avoid concerns about patients' perceptions of uterine activity, and the reports by Katz et al.[3-5] were promising. Perhaps overlooked in the development and deployment of this approach is the increased frequency of patient contact with the health care system, in this case the Term Guard nursing staff. The study design reported here allows comparison of clinical outcomes in two groups of high-risk women who all received frequent nursing contact. The groups differed primarily in the manner in which the uterine activity was assessed.

The principal end point for this study is the number of women reaching 35 and 37 weeks at delivery. We believe this is the most appropriate outcome for analysis since it represents the answer to the first question posed by the patient and her doctor when considering use of such a system: Will this approach prevent the birth of a premature infant? Other end points such as eligibility for tocolysis may be potentially misleading: If the contraction detection system does detect contractions and if contractions are the basis for the diagnosis, then of course the system is always 100% "successful." The addition of cervical examination findings is helpful, but they may also be highly subjective and interpreted differently according to the patient's previous obstetric history, when used to determine the initiation of tocolysis. All studies of preterm labor are affected by these uncertainties, so it seems best to place greatest emphasis on the least subjective end points such as gestational age and birth weight.

The similarity of all outcomes in groups E and EM is striking: The incidence of preterm labor, preterm delivery, and successful tocolysis is similar. Women using the monitor were not sent in for examination or treated prophylactically more often than those using self-palpation. Physicians who enrolled their patients in the study often forgot which study group the patient was in, suggesting that they perceived similarly the care received by both groups. The nursing staff seemed to move from a primarily contraction frequency–based approach in the early weeks of the study to a more global, symptoms-plus-contraction frequency–based approach as their experience grew. The similarity in outcome for groups E and EM indicates that advantages previously attributed to the monitored contraction data may in fact be the result of frequent nursing contact and careful attention to symptoms and perceived contractions. This in turn has implications for the prenatal care of women at increased risk of spontaneous preterm delivery: They should be educated about both preterm labor symptoms and contraction frequency as early markers of preterm labor and should be given ample opportunity to relate symptoms to a welcoming and supportive health care provider. We did not simultaneously enroll a third group of women who received a standard level of nursing and physician contact and therefore cannot draw any conclusions about whether frequent contact, with or without a monitor, is superior to current practice in reducing the incidence of preterm birth in high-risk patients. Such a study is now underway. We doubt, however, that a system based principally on contraction detection without equal attention to symptoms will be of use in reducing preterm births.

Another question unanswered by this study is the identification of specific subsets of the population at risk for preterm labor who might benefit most or least from tocodynamometer recording of uterine activity. Newman et al.[13] have reported increased levels of uterine activity among women with twins, which might make them more or less susceptible to any benefit of home tocodynamometry. Similar questions remain unanswered for women with uterine anomalies, incompetent cervices, and vaginal bleeding. Katz et al.[14] have also reported success with home monitoring in the management of oral tocolysis, with contraction frequency used as a guide to dosage adjustment, in 60 patients with preterm labor who used the Term Guard compared with 60 non–randomly selected matched control subjects. The study reported here specifically excluded

women treated for preterm labor before entry, but a similar prospective randomized trial of home monitoring in these patients is also underway.

In summary, this prospective random trial of intensive patient education and nursing support with and without a home uterine contraction monitor indicates that increased attention should be paid to the role of frequent and supportive patient contact in preterm birth prevention; the role of ambulatory contraction monitoring has yet to be defined.

We wish to express thanks to the physicians of central and southeast Ohio who enrolled their patients in this study, and to the Tokos Medical Corporation.

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Chronic oral terbutaline tocolytic therapy is associated with maternal glucose intolerance

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Plasma glucose determinations were performed 1 hour after a 50 gm oral glucose load in 30 patients receiving long-term terbutaline therapy (20 to 40 mg/day for at least 1 week) and 247 normal control patients. A total of 63% of patients receiving terbutaline had an abnormal 1-hour value (≥140 mg/dl), an incidence much higher than that of control subjects (17.8%) ($p < 0.0001$) for a relative risk of 3.54 (95% confidence intervals of 2.29 to 5.42). Mean 1-hour values were 112.1 mg/dl for control subjects and 149.8 mg/dl in the terbutaline group ($p < 0.0001$). All abnormal values were followed by a 3-hour 100 gm oral glucose tolerance test. A total of 15.9% of the glucose tolerance tests performed in the control group (2.8% overall) were abnormal as opposed to 52.6% (33.1% overall) in patients receiving terbutaline ($p < 0.01$). Nine patients were studied before and after terbutaline therapy. Results obtained during administration of terbutaline were significantly higher (102.2 mg/dl before therapy versus 145.2 mg/dl during therapy). We conclude that treatment with oral terbutaline appears to be associated with impairment of glucose tolerance in pregnancy. (Am J Obstet Gynecol 1987;157:644-7.)

Key words: Terbutaline, tocolysis, glucose intolerance

Treatment with intravenous β-sympathomimetic tocolytic agents can cause significant deterioration of maternal glucose homeostasis.[1, 2] We have previously shown that chronic oral ritodrine therapy does not produce significant clinical effects on glucose tolerance.[3] Over the last 2 years, we have largely switched to oral terbutaline therapy out of consideration for cost and dosing frequency. The effects of long-term (≥1 week) oral terbutaline therapy during pregnancy have not been studied in detail. The metabolic consequences of such treatment could be of critical importance because patients may require extended therapy with terbutaline at the time of greatest gestational insulin resistance, 26 to 30 weeks of gestation. This study was undertaken to determine the effects of long-term oral terbutaline therapy on carbohydrate tolerance in the early third trimester.

Subjects and methods

The study included all patients in our high-risk obstetric practice between January 1985 and July 1986. A 1-hour 50 gm oral glucose screening test was performed routinely at 26 to 30 weeks' gestation (92% of patients were studied between 27 and 29 weeks of gestation). The screening test was usually done in the morning when patients were fasting or after they had eaten a light meal. All plasma glucose levels that were ≥140 mg/dl after the screening test were considered abnormal and a 3-hour 100 gm oral glucose tolerance test was performed. The 3-hour glucose tolerance test was done in the morning after an overnight fast.

The diagnosis of gestational diabetes was made when two or more abnormal values were observed during the 3-hour glucose tolerance test. As recommended by the National Diabetes Data Group,[4] a fasting value of ≥105 mg/dl, a 1-hour value of ≥190 mg/dl, a 2-hour value of ≥165 mg/dl, and a 3-hour value of ≥145 mg/dl were considered abnormal. To further quantitate the degree of abnormality of the 3-hour glucose tolerance test, the area under the curve was roughly calculated by adding together half the fasting value, the 1- and 2-hour values, and half of the 3-hour value.[5] Patients with abnormal 3-hour glucose tolerance tests were given intensive dietary education. Patients with persistent fasting hyperglycemia (≥105 mg/dl) after 7 to 10 days of dietary management were treated with insulin.

Blood was obtained from all subjects by venipuncture and glucose determinations were performed on plasma samples by the glucose oxidase method. Patients were excluded from the study if they were known to have insulin-dependent diabetes or previously diagnosed gestational diabetes, if they were exposed to intravenous corticosteroids or β-sympathomimetics within 72 hours of testing, or if they were receiving β-sympathomimetic agents other than terbutaline.

Patients in the terbutaline group were treated with

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Table I. Group comparability

	Control	*Terbutaline*
No.	247	30
Age (mean ± SD) (yr)	31.9 ± 4.7	30.7 ± 3.7
Prepregnancy weight (mean ± SD) (pounds)	136 ± 29	135 ± 23
Family history of diabetes (%)	10.9	13.3
Gestational age at testing (wk)	28.7 ± 1.6	28.2 ± 2.3

Table II. Results of glucose tolerance testing

	Control	*Terbutaline*
Sample size	247	30
Proportion with abnormal 1 hr screen	44/247 (17.8%)	19/30 (63%)
	$p < 0.0001$*	
Proportion with abnormal 3 hr glucose tolerance test results	7/44 (15.9%)	10/19 (52.6%)
	$p < 0.01$*	

*χ^2 analysis.

20 to 40 mg/day in divided doses for at least 1 week before testing. Terbutaline therapy was continued during the glucose tolerance testing without change in dosing interval. There were five patients who had received corticosteroid treatment but none within 72 hours of glucose testing. The rate of abnormality in 1-hour glucose screening in these women (3/5 or 60%) was similar to that of the overall group (63%), so these patients were combined.

Birth weight was that recorded at delivery. Gestational age was based on the best obstetric estimate according to last menstrual period, ultrasonography (more than 90% of patients had at least one scan before 28 weeks' gestation) and serial office examinations. Birth weight index was calculated by dividing the measured birth weight by the fiftieth percentile birth weight. The fiftieth percentile weight was derived from a normal curve developed for patients in Philadelphia.[6]

Results were analyzed by Student's t test for continuous data (two tailed), χ^2 for proportions, and relative risk and confidence intervals as applicable.

Results

In total, 277 patients were studied, including 30 receiving oral terbutaline therapy for the prevention of recurrent preterm labor and 247 control subjects. Women treated with terbutaline and untreated women were comparable with respect to age (30.7 versus 31.9 years), prepregnancy weight, and history of diabetes in first-degree relatives (Table I). Only 6.6% of the treated women and 5.3% of the control women were less than 25 years of age. Gestational age at the time of testing was also similar between treated and untreated patients (28.2 ± 2.3 versus 28.7 ± 1.6 weeks). All comparisons were statistically insignificant at the $p > 0.05$ level by Student's t test or χ^2 analysis.

The proportions of women who had an abnormal 1-hour screening test and an abnormal 3-hour glucose tolerance test on follow-up testing are reported in Table II. In the untreated group, 44/247 or 17.8% had an abnormal screening test while 19/30 or 63% of the terbutaline-treated group had values ≥140 mg/dl ($p < 0.0001$, χ^2 analysis). The relative risk (odds ratio) for an abnormal 1-hour screening test during terbutaline administration compared with that for control women is 3.54 with 95% confidence intervals of 2.29 to 5.42. Thus we would anticipate that at least 40.8% of women receiving long-term oral terbutaline therapy would have an abnormal 1-hour screening test.

Of the 3-hour oral glucose tolerance tests, results were abnormal in 7/44 (15.9%) of the control women compared with 10/19 (52.6%) in the terbutaline group ($p < 0.01$, χ^2 analysis). Overall rates of gestational diabetes were 2.8% in control women but 33% for the terbutaline group. Furthermore, five of the 10 (50%) patients with gestational diabetes who were given terbutaline had persistent fasting and postprandial hyperglycemia and were treated with insulin therapy. Two of seven (29%) patients with gestational diabetes in the control group also required insulin.

Mean 1-hour screening values for the terbutaline and control groups are illustrated in Fig. 1. The control population in the current study had a mean plasma glucose level of 112.1 ± 25.6 mg/dl while the terbutaline group had a significantly higher mean of 149.8 ± 29.9 mg/dl ($p < 0.0001$). We have previously studied the effects of ritodrine with an identical study design,[3] and these results are also shown in Fig. 1. The mean plasma glucose values for the control and ritodrine populations in that study were similar (117.7 and 117.0 mg/dl).

In the current study, the calculated area under the 3-hour glucose tolerance test curve was nearly 100 mg/dl greater in the terbutaline group than in the control women (493.5 ± 124.4 versus 395.7 ± 68.8). This was highly significant at $p < 0.0001$ (Student's t test).

We studied nine patients before and during terbutaline therapy. Terbutaline treatment was administered as described for the larger group (20 to 40 mg/day, duration at least 1 week before testing). These nine patients had a routine screening test at 27 to 28 weeks and then were restudied after being placed on a regimen of terbutaline. The median interval between tests was 2.3 weeks (range 1.1 to 4.2 weeks). As illustrated in Fig. 2, each patient experienced a rise in the 1-hour screening glucose value. The mean 1-hour plasma glu-

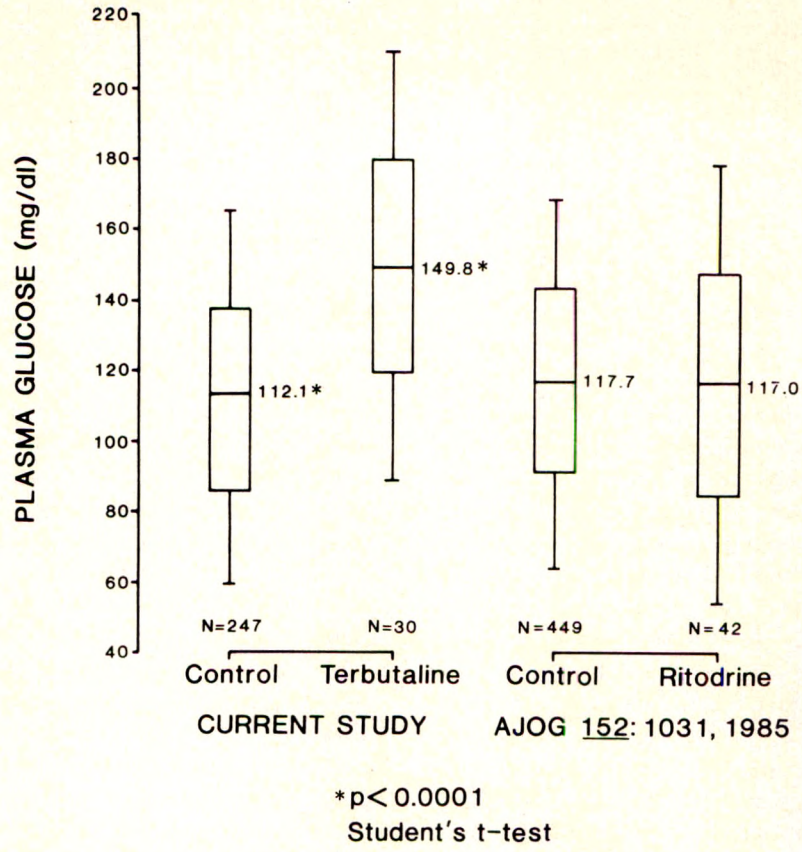


Fig. 1. Screening test results 1 hour after 50 gm of glucose in control and terbutaline-treated patients (mean ± 1 and 2 SD). Our previous results[3] with control and ritodrine patients are illustrated on the right (also mean ± 1 and 2 SD).

Table III. Infant size

	Control (mean ± SD)	*Terbutaline (mean ± SD)*
Gestational age (wk)	39.1 ± 1.6	37.0 ± 1.9
	$p < 0.0001$*	
Birth weight (gm)	3341 ± 461	3101 ± 682
	$p < 0.024$*	
Birth weight index†	1.05 ± 0.13	1.10 ± 0.17
	$p = 0.14$*	

*Student's t test.

†Calculated by dividing the measured birth weight by the fiftieth percentile of birth weight (derived from a normal curve for patients in Philadelphia[6]).

cose level before terbutaline was 102.2 ± 36.1 while the mean value during terbutaline therapy was 145.2 ± 25.4 ($p < 0.0001$) (paired *t* test). Two of the nine women had abnormal glucose screening results before terbutaline therapy, but both had a normal 3-hour glucose tolerance test thereafter. After the initiation of terbutaline, results of five of nine glucose screening tests became abnormal and two of these women exhibited gestational diabetes on the 3-hour oral glucose tolerance test. These results are similar to those of the larger group.

We also assessed whether chronic terbutaline therapy was associated with larger birth weights (Table III). This analysis is complicated by the fact that terbutaline was used because the patients had experienced preterm labor and hence were at greater risk for preterm birth. Indeed the mean gestational age in the terbutaline group was 2.1 weeks less than that of the control women (37.0 ± 1.9 versus 39.1 ± 1.6 weeks). The mean birth weight was 240 gm less, a somewhat smaller difference than might be expected for this gestational age difference. Therefore we corrected for gestational age by using the birth weight index. With this correction, there was a tendency toward bigger babies in the terbutaline group but this did not reach statistical significance ($p = 0.14$).

Comment

In our two studies of long-term oral tocolytic therapy, we have noted a clear difference in the effects of terbutaline and ritodrine on glucose tolerance during pregnancy. This is not without precedent. Caritis et al.[7] compared intravenous ritodrine and terbutaline in a

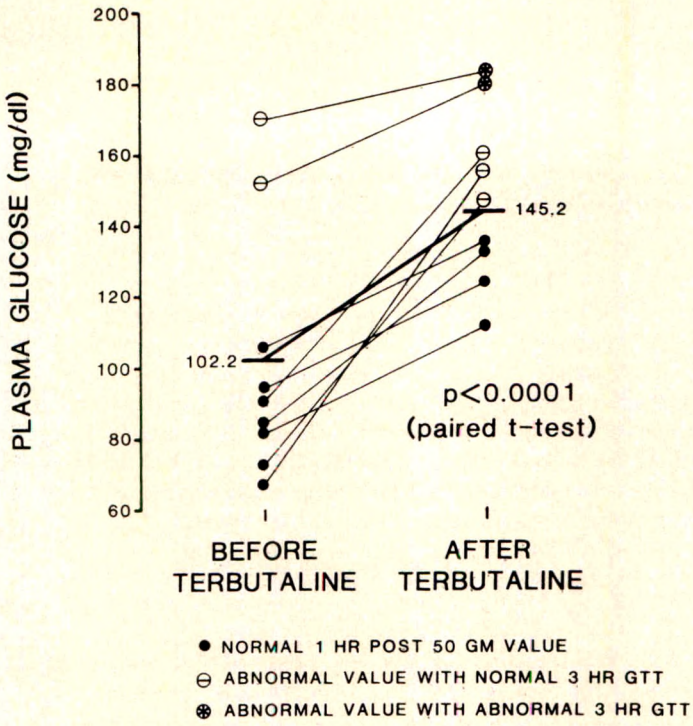


Fig. 2. Results of glucose tolerance testing in nine patients before and during oral terbutaline therapy.

randomized blinded trial. They noted that patients receiving terbutaline had a significantly greater chance of developing hyperglycemia than those receiving ritodrine.

Why should there be a difference between terbutaline and ritodrine? First, terbutaline appears to be a less selective β_2-adrenergic receptor agonist than ritodrine. Second, different tissues may have different sensitivities to various β-sympathomimetics. Which of these or other mechanisms is important for glucose tolerance during pregnancy remains to be determined by more intensive study.

While we could not detect any clinical differences between terbutaline and control groups in terms of outcomes this would not be expected with our study design. All patients were screened and, if appropriate, treated. Thus the natural history and consequences of maternal hyperglycemia were not apparent in this investigation.

As our sample size is small, we can be less certain as to the actual magnitude of the effect of terbutaline on glucose tolerance. However, from our data analysis (odds ratio and confidence intervals) we would expect that there is a 95% likelihood that a minimum of approximately 40% of patients receiving terbutaline will have abnormal 1-hour 50 gm glucose screens with at least a proportionate increase in gestational diabetes. These findings indicate that all pregnant patients receiving long-term terbutaline therapy should undergo screening for glucose intolerance at least once after 7 days of terbutaline therapy. Furthermore, these results underscore the need for careful assessment of the potential risks and benefits of long-term oral tocolytic therapy.

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Fetal echocardiographic screening for congenital heart disease: The importance of the four-chamber view

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The four-chamber view of the heart has been proposed as a screening method for obstetrical sonographers to assess the fetus for the presence of congenital heart disease. We examined the fetuses in 1022 pregnancies and found 74 structurally abnormal hearts. Seventy-one of these (96%) were found to have an abnormality as seen in the four-chamber view. The four-chamber view of the heart had 92% sensitivity and 99.7% specificity in the detection of congenital heart disease. The positive predictive value was 95.8%, and the negative predictive value was 99.4%. We conclude that the four-chamber view may be a useful screen for the presence of congenital heart disease and should be included as a part of all routine obstetric ultrasound examinations. (Am J Obstet Gynecol 1987;157:648-55.)

Key words: Congenital heart disease, ultrasound, fetal diagnosis

Fetal echocardiography is an accurate tool for the prenatal diagnostic evaluation of the fetus at risk for congenital heart disease.[1-5] Pregnancies with any of a number of risk factors should be screened to identify affected fetuses, and the information obtained should be used in the formulation of perinatal and neonatal management plans.[6] Additionally, fetuses with arrhythmias may be evaluated and treatment of certain arrhythmias may be offered based on these techniques for diagnosis[7] and monitoring therapy.[8]

Despite attempts to identify high-risk groups, most neonates with congenital heart disease do not have prenatal risk factors. Thus a method to permit the clinician to screen for congenital heart disease during routine sonographic examinations would be useful. The four-chamber view of the fetal heart has been proposed for this purpose.[9, 10]

We have reported that the incidence of congenital heart disease among fetuses referred for fetal echocardiography due to a suspicion of cardiac abnormality during a routine obstetric scan is 50%.[6] We here present the results of a prospective and retrospective evaluation of the appearance of the four-chamber view in a group of fetuses at high risk of congenital heart disease who were scanned during the second and third trimesters of pregnancy over a 3-year period.

Material and methods

Between January 1, 1984 and December 31, 1986, a total of 1193 fetal echocardiograms were performed on 991 women carrying a total of 1022 fetuses. There were 31 sets of twins, two sets of conjoined twins, and one normal twin with an acardiac, acephalic parasitic co-twin. Indications for examination (Table I) were in accordance with previously identified selection criteria that placed the mothers at risk for carrying fetuses with heart disease.[11] Gestational ages at the time of diagnosis varied from 18 weeks to term.

All patients underwent a detailed biometric examination followed by fetal echocardiography with 5 mHz sector scanners (Hewlett-Packard Model 77020A, Andover, MA; Advanced Technologies Laboratories model MK600, Bellvue, WA; Technicare model 256, New Brunswick, NJ). Fetal echocardiographic examinations consisted of standard tomographic views (four-chamber, short axis of the ventricles and great vessels, long axis of the left ventricle, aortic arch, and ductus)[11] for all patients. Pulsed Doppler examinations were carried out on selected patients for the evaluation of dysrhythmias and valvular competence with equipment calibrated by the manufacturers to comply with applicable Food and Drug Administration acoustic output limitations (model MK 600 and UltraMark 4, Advanced Technologies Laboratories; model RT 3600, General Electric, Milwaukee, Wisconsin).

All structural diagnoses were based on the appearance of the heart on two-dimensional scan. M-Mode

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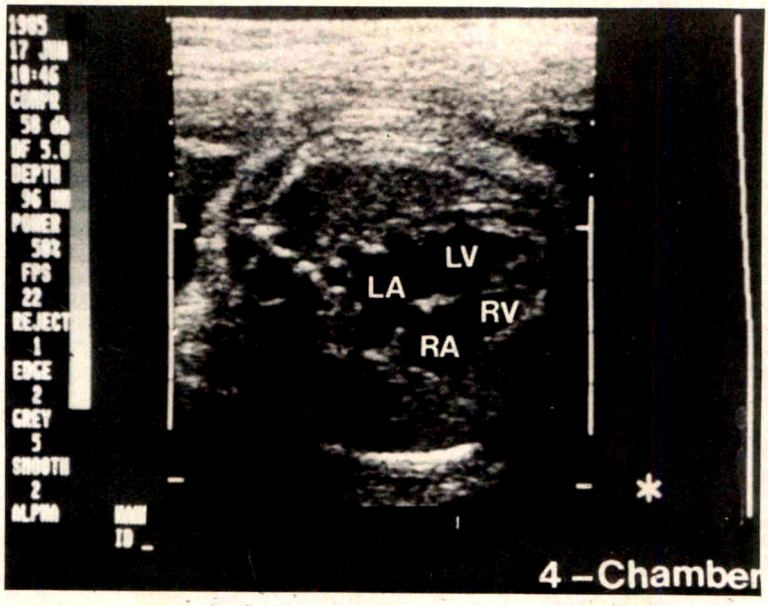


Fig. 1. Normal four-chamber view of the heart. The left atrium *(LA)* lies most posteriorly. The right atrium and ventricle *(RA, RV)* are close to the chest wall anteriorly. *LV* = left ventricle.

Table I. Indications for fetal echocardiography

Indication	*Patients*		*Patients with congenital heart disease*	
	n	%	*n*	%
Family history	310	30.3	3	1
Previous child	215		3	1.4
Maternal	49		0	
Other	46		0	
Arrhythmias	182	17.8	3	1.6
Isolated extrasystole	159		2	1.3
Tachycardia	9		0	
Bradycardia	14		1	7.1
Extracardiac anomalies	117	11.4	27	23.1
Cardiac teratogens	101	9.9	2	2
Maternal diabetes	96	9.4	3	3.1
Suspicious level I scan	40	3.9	20	50
Aneuploidies	28	2.7	7	25
Nonimmune hydrops	27	2.6	9	33.3
Other	121	11.8	0	
Total	1022	100	74	7.24

echocardiography was used only for the evaluation of rhythm disturbances. All scans were performed and/or reviewed by at least two physicians experienced in fetal cardiac ultrasound. Scans were recorded on videotape for later review. Follow-up information was obtained from clinical or autopsy records from Yale-New Haven Medical Center or by contacting the referring physicians.

The four-chamber view of the heart was obtained as the initial step of each fetal echocardiogram. The abdominal circumference was visualized and the transducer was directed toward the fetal head. The four-chamber view was considered to be normal if the two atria and two ventricles were equal to each other in size and contractility, and if none of the abnormal criteria were present (Fig. 1). The four-chamber view of the heart was considered to be abnormal if any of the following were present: ventricular disproportion, myocardial hypertrophy, dilation or hypoplasia of any of the chambers, septal defects other than the presence of the foramen ovale, or obvious deformities of the atrioventricular valves. These assessments were based on an evaluation of the two-dimensional scan, and not on M-mode measurements.

Table II. Lesions identified

Lesion	No.	Abnormal four-chamber view	
		n	%
Complete atrioventricular septal defect	16	16/16	100
Hypoplastic left heart syndrome	8	8/8	100
Ventricular septal defect	7	5/7	71
Pulmonic stenosis	6	6/6	100
Ebstein anomaly	5	5/5	100
Complex lesions	5	5/5	100
Tetralogy of Fallot	5	5/5	100
Ectopia cordis (pentalogy of Cantrell)	4	4/4	100
Atrial isomerism variants	3	3/3	100
Double outlet right ventricle	3	3/3	100
Isolated pericardial effusions	2	2/2	100
Conjoined twins	2	2/2	100
Acardiac acephalic parasite	1	1/1	100
Situs inversus, isolated levocardia*	1	1/1	100
Others†	6	5/6	83
Total	74	71/74	96

*Structurally normal heart.

†Includes one each: tubular heart, small foramen ovale, pulmonic regurgitation, false positive identification of aortic coarctation, subaortic stenosis, and duplication of aorta (last was only one with normal four-chamber view in this group).

Table III. Incorrect cardiac diagnoses

	Four-chamber view
False negative	
Coarctation of aorta	Normal
Transposition of great vessels	Normal (2 cases)
Tricuspid valve tumor	Normal
Tetralogy of Fallot (mild)	Abnormal
False positive	
Subaortic stenosis	Abnormal
Coarctation of aorta	Abnormal
Double outlet of right ventricle	Abnormal
Aortic duplication	Normal

Table IV. Diagnostic indices of four-chamber view

True positive	69
False positive	3
False negative	6
True negative	944
Sensitivity	69/75 = 92%
Specificity	944/947 = 99.7%
Positive predictive value	69/72 = 95.8%
Negative predictive value	944/950 = 99.4%

Results

A total of 74 cardiac anomalies were identified. Table I demonstrates the diagnostic groups from which these patients originated. Hypertrophic cardiomyopathy among fetuses of mothers with diabetes is not included in these data. Pericardial effusions in patients with nonimmune hydrops fetalis and otherwise structurally normal hearts are also not included.

Follow-up data have been obtained on all fetuses suspected to have cardiac anomalies. The prenatal cardiac diagnosis was correct in all but nine cases. There were five false negative and four false positive diagnoses. In several of the false positive diagnoses, the four-chamber view of the heart was normal.

False negative results. One fetus had an apparently normal heart at 21 weeks' gestation, but was found at birth to have right ventricular inflow obstruction, right ventricular hypoplasia, and right-to-left atrial shunting. The right ventricular inflow obstruction was attributed to a neoplasm of the tricuspid valve. Review of the prenatal and postnatal echocardiograms suggested that the lesion had been acquired between 20 and 40 weeks' gestation.

A fetus scanned at 29 weeks because of a fetal arrhythmia and found to have atrial extrasystole was diagnosed after delivery to have transposition of the great vessels. Review of the scan demonstrated suboptimal visualization of the great vessels and normal four-chamber anatomy.

Another fetus scanned at 19 weeks due to a fetal arrhythmia (determined to be atrial extrasystole) was found to have coarctation of the aorta after delivery. Review of the prenatal scan confirmed the normal appearance of the four-chamber view of the heart.

A woman with White Class D diabetes, in good control through the first trimester (hemoglobin A_{1c} <6 gm %), was scanned at 18 weeks' gestation. Although the scan was read as normal at the time, the neonate was cyanotic, and a postnatal echocardiogram demonstrated complete transposition of the great arteries. Review of the prenatal scan confirmed a normal four-chamber view, with poor visualization of the great arterial courses.

The final false negative diagnosis occurred in a patient scanned at 20 weeks because of maternal tetralogy of Fallot. She had undergone surgical repair in childhood and was not cyanotic. Despite a normal fetal echocardiogram, pulmonic stenosis and a perimembranous ventricular septal defect were detected at birth. Retrospective review of the echocardiogram confirmed the abnormal appearance of the heart, including an abnormal four-chamber view demonstrating ventricular disproportion.

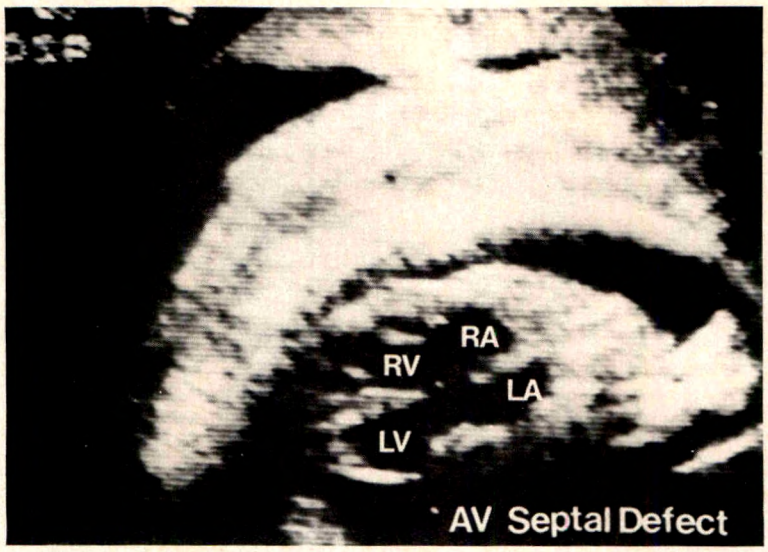


Fig. 2. Four-chamber view of heart with complete atrioventricular septal defect. The large defect between the atria and ventricles is readily apparent. A large pleural effusion is also present. *LA* = Left atrium; *RA* = right atrium; *LV* = left ventricle; *RV* = right ventricle.

False positive results. A fetus scanned at 19 weeks for partial trisomy 7 was suspected to have a coarctation of the aorta, based on ventricular disproportion (right ventricular dilation). Although the great vessels were normal on postmortem examination, right ventricular hypertrophy was confirmed.

Another fetus was scanned at term because of a "fetal heart murmur," with a ventricular septal defect suspected from a scan at another institution. The interventricular septum appeared to be intact, but the aorta was extremely tortuous, and an aortic arch anomaly was suspected because of the apparent redundancy of the vessel. After delivery, echocardiography demonstrated a normal heart with a tortuous, but otherwise normal, aorta. The four-chamber view, however, was normal.

A fetus scanned at 26 weeks because of polyhydramnios and a suspicious appearance of the heart during obstetric evaluation of the amniotic fluid was suspected to have subaortic stenosis. The polyhydramnios progressed to oligohydramnios with intrauterine growth retardation. Amniocentesis revealed Klinefelter syndrome (47,XXY). Continuing echocardiographic examination results did not change. Postnatal echocardiography demonstrated an elongated left ventricular outflow tract, with no stenosis.

The final false positive study was in a fetus scanned at 21 weeks before elective termination for Down syndrome. The four-chamber view was abnormal because of biventricular dilation, and evaluation of the outflow tracts suggested overriding aorta. Results of postmortem examination of the heart were normal.

Determination of congenital heart disease. Table II summarizes the cases of congenital heart disease encountered during the course of this investigation. Seventy-one of the 74 (96%) fetuses identified to have congenital heart disease had abnormalities potentially detectable in the four-chamber view of the heart. In some cases the anatomic defect could not be completely delineated from the four-chamber view alone. For example, severe pulmonic stenosis (encountered in two fetuses) caused dilation of the right ventricle and atrium in each. Further assessment, including two-dimensional views of the right ventricular outflow tract and pulsed Doppler flow studies, clarified the diagnosis.

The false negative and false positive diagnoses that occurred are summarized in Table III. The diagnostic utility of the four-chamber view is analyzed in Table IV. For the latter, only the pathologically proved cases of congenital heart disease are considered.

Although pericardial effusions in hydropic fetuses were excluded from study, two patients with final diagnoses of isolated pericardial effusion are included. One was referred because of hydrocephalus and later underwent amniocentesis, revealing a ring chromosome 13. Except for pericardial effusion, the heart was normal at autopsy. The other was referred because of an abnormal four-chamber view of the heart, with an isolated pericardial effusion noted at 22 weeks. This resolved over the next several weeks, and at 28 weeks hydrocephalus was noted. Congenital viral infection was diagnosed in the neonatal period based on hydrocephaly and periventricular calcifications. Neonatal serology studies were not diagnostic.

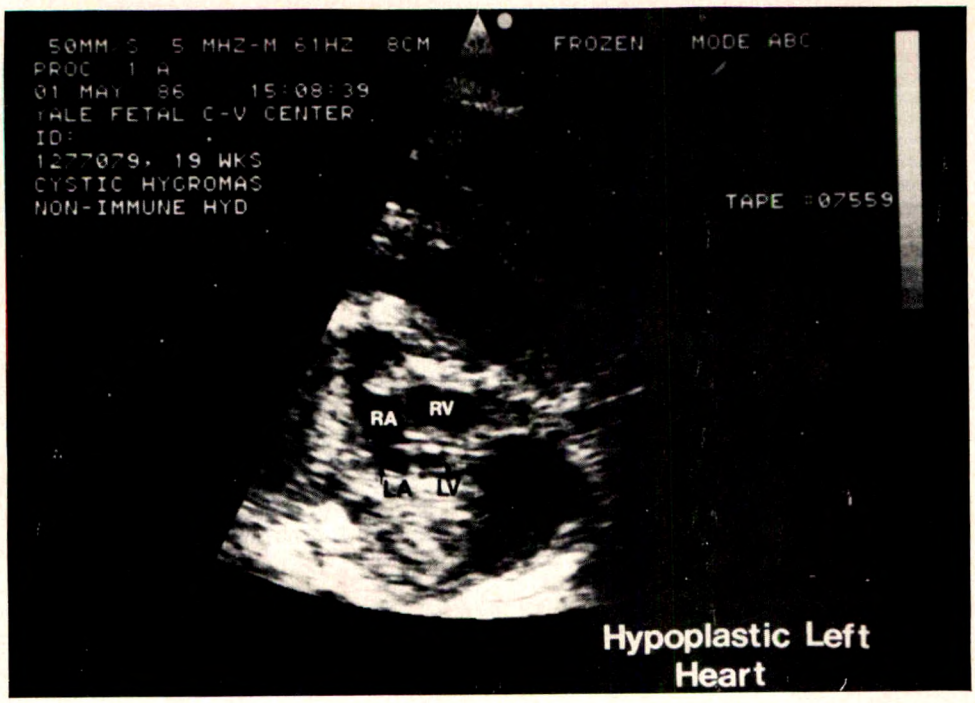


Fig. 3. Four-chamber view of the heart with hypoplastic left heart syndrome. The fetus presented with nonimmune hydrops and cystic hygromas at 19 weeks. Karyotype confirmed Turner syndrome (45,X).

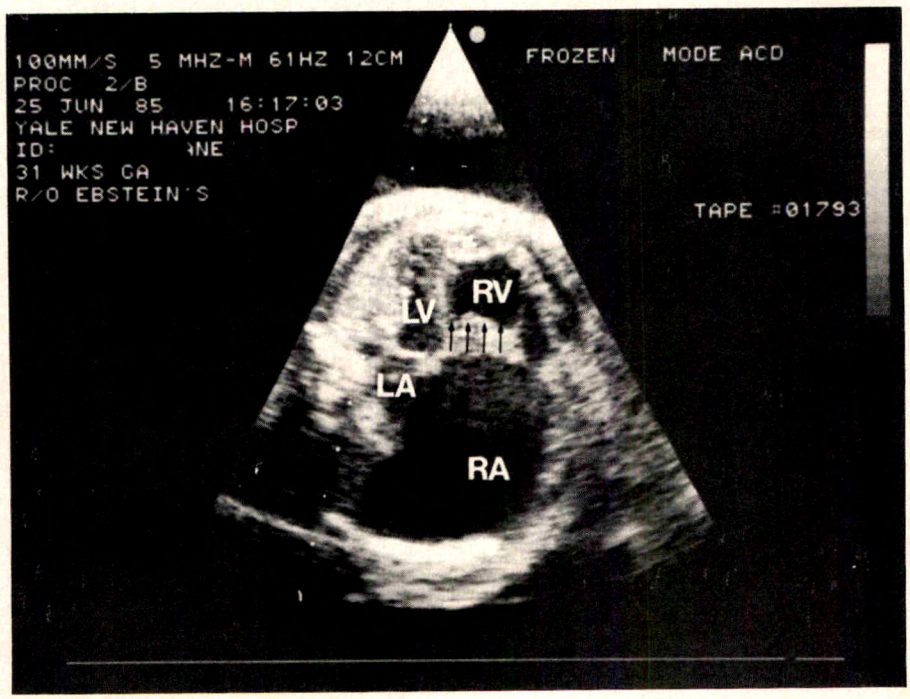


Fig. 4. Four-chamber view of a heart with Ebstein malformation of the tricuspid valve. There was no history of lithium exposure. The right atrium *(RA)* is greatly enlarged, with a much smaller left atrium *(LA)* adjacent to it. The malformed tricuspid valve is shown with small *arrows*. *LV* = Left ventricle; *RV* = right ventricle.

Atrioventricular septal defects (16 cases), hypoplastic left heart syndrome (eight cases), and Ebstein anomaly of the tricuspid valve (five cases) constituted 29 of the 74 anomalies encountered (39%). These were easily diagnosed on the four-chamber view (Figs. 2 to 4).

Comment

The four-chamber view of the heart is relatively easy to obtain during routine obstetric sonography. By tilting the transducer cephalad from the tomographic plane used to measure the abdominal circumference, one brings the four cardiac chambers into view. As such, it is simple to include as a screening view for congenital heart disease, and our experience has suggested that is obtainable in >95% of cases scanned between 18 weeks and term.

We have demonstrated that some abnormality of the four-chamber view of the heart was present in 69 fetuses encountered during the study period who were subsequently found to have congenital heart disease. There are potential problems with our data: (1) The data are drawn from patients referred for fetal echocardiography and thus at high risk for congenital heart disease; (2) we are experienced in fetal echocardiography and thereby particularly sensitized to the need for detailed examination of the heart; (3) although the sensitivity of the four-chamber view of the heart to the presence of congenital heart disease was 92% in this series, the data are insufficient to allow us to comment on the sensitivity of the four-chamber view of the heart when performed as part of a "level I" scan by less experienced fetal cardiac sonographers.

The sensitivity of the four-chamber view to the presence of congenital heart disease was not perfect. This has been the experience of other centers as well.[12] The lesions detected with a high degree of sensitivity are those that are expected to distort the four-chamber anatomy. We are less confident in our ability to detect lesions that do not affect that view. This point is important, because a screening four-chamber view cannot be offered to patients as a means of ruling-out all forms of congenital heart disease. Rather, it is an effective screen for many of the more severe forms of congenital heart disease, for which the prognosis might be improved by controlled delivery at a center equipped to manage the neonate. The ability of a four-chamber view to exclude congenital heart disease is attested to by the high negative predictive value of the test (99.4%).

It is of interest that 20 of 40 (50%) patients referred because of a suspicious level I scan were found by fetal echocardiography to have congenital heart disease, suggesting a relatively high specificity. This may be compared with an incidence of 5.5% (54/982) among fetuses referred for all other indications and is consistent with our previous preliminary report[6] of a portion of the patients reported upon here, in which a 50% incidence of congenital heart disease was found among such patients. Allan et al.[10] have noted a similar increase in the proportion of referrals because of suspected abnormalities in screening ultrasound studies. Preliminary data from a prospective study involving screening four-chamber views of the heart suggest that a large proportion of structural cardiac disease may be detected in utero.[12]

We have intentionally not used M-mode measurements of the heart because they are more difficult to obtain and require specialized training to apply accurately. We have found, moreover, that the orientation of the fetal heart is often not conducive to cursor placement for such measurements. In choosing to evaluate our data based on subjective criteria, we were attempting to use a more widely applicable standard, recognizing that the experience of the sonologist will play a part in any such criteria.

Table V. Proposed indications for fetal echocardiography

Fetal risk factors
Extracardiac anomalies
Chromosomal
Anatomic
Fetal cardiac arrhythmia
Irregular rhythm
Tachycardia (>200 bpm)
Bradycardia (nonperiodic)
Nonimmune hydrops fetalis
Suspected cardiac anomaly on level I scan
Maternal risk factors
Congenital heart disease
Cardiac teratogen exposure
Lithium carbonate
Progestins
Amphetamines
Alcohol
Anticonvulsants
Phenytoin
Trimethadione
Isotretinoin
Maternal metabolic disorders
Diabetes mellitus
Phenylketonuria
Polyhydramnios
Maternal infections
Rubella
Toxoplasmosis
Coxsackie virus
Cytomegalovirus
Mumps
Familial risk factors
Congenital heart disease
Previous sibling
Paternal
Syndromes
Noonan
Tuberous sclerosis

Certain types of congenital heart disease can be anticipated not to cause abnormalities of the fetal four-chamber view. These would include simple small perimembranous or muscular ventricular septal defects and simple transposition of the great arteries. These lesions are frequently accompanied by other intracardiac abnormalities that might cause abnormalities of the four-chamber view.[13, 14] While coarctation of the aorta may be found in association with disproportionate right ventricular dilation, reflecting altered fetal flow patterns, it is not clear whether this is seen in early gestation. Our experience has also suggested that right ventricular dilation may be found in a variety of pathologic states, and that direct identification of a "shelf" of obstructing aortic tissue may be difficult, if not impossible in utero.

High-risk gravid women should still undergo more detailed fetal echocardiographic examinations with multiple overlapping tomographic views to maximize the identification of structural abnormalities, even if the four-chamber view is normal. Because the technology involved in obtaining a detailed complete fetal echocardiographic study is complex and expensive and precise diagnosis requires specialized knowledge of congenital cardiac malformations, certain indications have been suggested to target populations at particular risk of congenital heart disease. The overall population risk of a neonate having congenital heart disease is 0.8%.[15] If a previous sibling has been born with congenital heart disease, however, this rises to 2% to 3%,[16] and if the mother herself has congenital heart disease, the risk may be as high as 10% for her offspring.[17] Other identified risk groups are listed in Table V. For those pregnancies at high risk of congenital heart disease based on any of these factors, complete detailed fetal echocardiography should continue to be offered.

The distribution of indications for sonography (Table II) should not be taken as representative of the general obstetric population, but rather as a reflection of the referral patterns to our institution. Some patients fell into more than one group, such as patients referred for a suspicious screening examination who were then found to have hydrops, or patients found to have chromosomal abnormalities after the cardiac evaluation. In these situations, only the original referral indication is listed. For this reason, Table II cannot be used to estimate the prevalence of such conditions among patients with cardiac anomalies, or the frequency of cardiac abnormalities among all patients with the conditions.

Similarly, the distribution of cardiac diagnoses differs from that found in pediatric series.[18] This may be due to a number of factors, but probably reflects a bias of the technique toward the identification of lesions altering the four-chamber anatomy. It also suggests that many of the fetuses identified antenatally may not survive to the point of inclusion in pediatric series.

We conclude on the basis of our 36-month survey that a carefully performed level I obstetric ultrasound scan should include a four-chamber view of the fetal heart. The sensitivity of such a scan for the detection of congenital heart disease will not be perfect, with anticipated weaknesses including a lack of detection of uncomplicated ventricular septal defects, mild semilunar valve stenoses, aortic coarctation, and simple transposition. However, it can be used to detect anomalies that directly affect the structure of the atrioventricular junction, such as atrioventricular septal defects or Ebstein malformation of the tricuspid valve. In addition, lesions imparting ventricular hypoplasia should be readily identified, especially if these anomalies are associated with atrioventricular valve atresia. Other lesions that cause a greater than normal disparity in ventricular dimensions (e.g., severe semilunar obstruction and/or semilunar or atrioventricular valve insufficiency) or pericardial effusions should be easily identified on such scans.

Our experience has suggested that including a view of the fetal heart in the level I scan will improve the identification of fetuses with congenital heart disease. While it cannot replace the detailed echocardiogram for the fetus at high risk for congenital heart disease, routine application of this simple additional view in the basic ultrasound examination should help to decrease the incidence of undetected congenital heart disease at birth. The sensitivity and specificity we report reflects in part the prevalence of disease in the population under study. Confirmation of these results must be obtained from further prospective studies of the true potential for the use of the four-chamber view as a general screening test. Programs have already been undertaken to implement such screening in Europe and England.[10, 12] Although ultrasonographic examination has not been accepted as a routine part of obstetric care in the United States, a large proportion of prenatal patients are currently scanned. If scans are performed in the second or third trimester of pregnancy, a four-chamber view of the heart should be included. Abnormalities should prompt referral to a facility experienced in detailed evaluation of the structure of the fetal heart.

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Ultrasound evaluation of tachycardia-induced hydrops in the fetal lamb

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Nonimmune hydrops was induced in six fetal lambs at 120 days' gestation by right atrial pacing at 300 beats/min. Biochemical, hemodynamic, and Doppler parameters were observed and monitored by real-time ultrasound during the creation (18 to 42 hours) and resolution of hydrops. The significant changes seen were hypoproteinemia (34.3 to 29.0 mg/100 ml), venous hypertension (24.6 to 31.3 mm Hg), decreases in thoracic aorta flow, and decreases in systolic/diastolic ratios in the aorta and umbilical artery. These changes were reversed with the discontinuation of pacing and the resolution of fetal hydrops. (Am J Obstet Gynecol 1987;157:655-9.)

Key words: Pacing-induced hydrops, fetal tachycardia

Cardiovascular disease has been implicated as a significant cause of nonimmune fetal hydrops.[1] Within this group of disorders supraventricular tachycardia has come into greater focus, because it is amenable to maternal administration of antiarrhythmic drugs.[2, 3] As a result there is a need for a clearer understanding of the natural history and the changes that occur during the evolution of nonimmune hydrops. Prenatal diagnosis of hydrops by ultrasound has been well established in the human fetus.[4] However, investigators working with the fetal lamb hydrops model have been able to assess this condition only at autopsy.[5]

This study assessed the real-time and Doppler ultrasound changes that occurred during the production and resolution of fetal hydrops caused by right atrial pacing.

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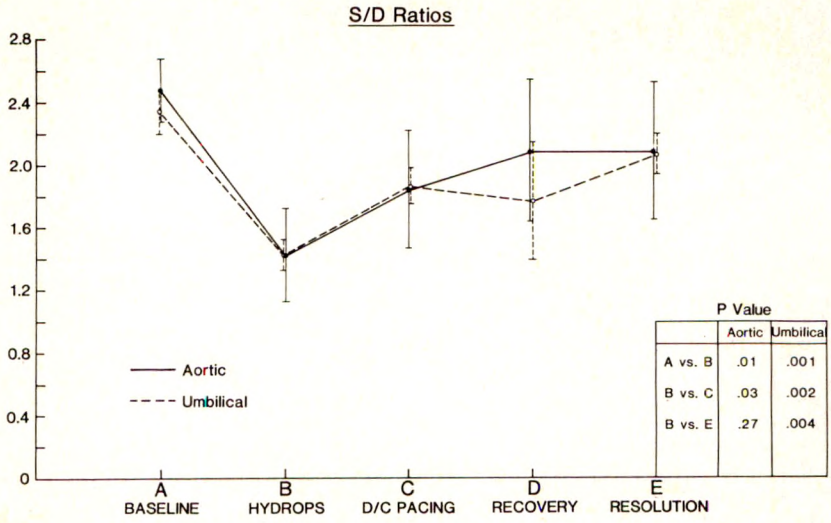


Fig. 1. Changes in aortic and umbilical systolic-diastolic *(S/D)* ratios of fetal lambs with levels of statistically significant changes included.

Table I. Biochemical parameters in six fetal lambs

	Baseline	*At hydrops*	*At resolution*
Potassium	4.6 ± 0.7	4.7 ± 0.7	4.3 ± 0.3
Sodium	149.8 ± 4.0	144.1 ± 2.6	147.8 ± 1.3
Protein	34.3 ± 3.8	29.0 ± 2.6*	32.6 ± 1.9
Albumin	23.0 ± 2.6	19.5 ± 1.3*	22.5 ± 1.6
pH	7.33 ± 0.02	7.33 ± 0.02	7.34 ± 0.02
P_{O_2}	18.2 ± 4.2	18.6 ± 3.6	20.7 ± 4.2
P_{CO_2}	42.2 ± 5.1	46.7 ± 2.1	43.4 ± 3.9

*$p < 0.05$.

Methods

Preparation. Six pregnant ewes and their fetuses at approximately 115 days' gestation (term is 143 days) were surgically instrumented and studied. Each ewe was anesthetized by an intravenous injection of 5% sodium thiopental. After they were intubated anesthesia was maintained with a halothane-oxygen mixture. Under sterile surgical technique, the uterus was exposed via a midline incision and the fetal head and chest were exposed. An incision was made in the fourth intercostal space from the right side to expose the heart, and the pericardial sac was opened. The great vessels were identified, and bipolar pacemaker electrodes were implanted in the right atrial appendage close to the insertion of the superior vena cava. The pericardium was left open when the chest was closed. Electrocardiographic electrodes were implanted under the skin of the fetus, with one lead placed in the neck and the other lead placed in the chest wall at the sixth rib.

Fetal femoral artery and jugular venous catheterization was performed. The lost amniotic fluid was replaced by sterile saline, and the uterine incision was closed. The catheter and leads were exteriorized through the ewe's flank before the midline skin incision was closed. Prophylactic antibiotics were administered, and the animals were allowed to recover from the anesthesia. Experiments took place after a minimum recovery period of 4 days.

Experimental protocol. All control and experimental studies were conducted while the ewe was awake and lying restrained on a specially constructed, cushioned table. Between study times the animals were allowed mobility with normal food and water. Baseline biochemical and cardiovascular data were obtained during a control period when measurements were allowed to stabilize for at least 30 minutes. The fetal pacemaker leads were attached to a General Electric Standby Pacemaker 7118 (General Electric, Fairfield, Connecticut), and pacing occurred at 300 beats/min from a mean baseline of 178 beats/min. Pulsed Doppler studies were done during periods of absent fetal breathing by use of a Toshiba 3.5 MHz linear-array transducer combined with a 2.42 MHz fixed-angle pulsed Doppler system with a 100 Hz thump filter. The fetal descending thoracic aorta, umbilical artery, and inferior vena cava were studied. The ultrasound machine provided online calculations of flow velocity and volume flow from the descending aorta. The peak systolic and end-diastolic velocities and systolic/diastolic ratios of the fetal aorta and umbilical artery were calculated by off-line averaging of the measurements from three consecutive waveforms.

After baseline biochemical and ultrasound parameters were measured, pacing was commenced. After the first 12 hours of pacing, real-time ultrasound and blood sampling for biochemical analysis were repeated at ap-

Table II. Age, duration of pacing, and resolution time in six fetal lambs

Animal	*Gestational age at surgery (days)*	*Gestational age at pacing (days)*	*Time to hydrops (hr)*	*Time to clearance (hr)*
1	107	114	41	31
2	116	123	18	18
3	113	118	23	50
4	120	124	40	24.5
5	114	126	27	29.5
6	115	119	22.5	36
Mean	114.2 ± 4.3	120.7 ± 4.5	28.6 ± 9.6	31.5 ± 10.9

proximately 4-hour intervals until the ultrasound confirmation of fetal hydrops was made. Hydrops was considered to be present when marked fetal ascites was present with a rim of fluid greater than 1 cm all around the abdomen. This was much easier to recognize and quantitate than pleural effusion or skin edema, and hence it was the only criterion used.

For the purpose of analysis data were presented at the following intervals: (1) before pacing commenced, (2) at the ultrasound diagnosis of fetal hydrops, (3) immediately after pacing was discontinued, (4) during recovery at 12 to 16 hours after pacing was discontinued, and (5) at resolution of fetal hydrops (no ascites or pleural effusion visible on ultrasound).

The ewe was removed from the cushioned table to a metabolic cage after each evaluation. Statistical analysis of data was done with a paired *t* test for grouped results.

Results

Each of the six animals was considered to be normal on the basis of the baseline blood gases, electrolytes, blood pressure, and mean heart rates at the commencement of each study. Table I highlights these baseline values and the biochemical changes seen with the development and clearance of fetal hydrops. There was no statistically significant change in sodium and potassium concentration during development of fetal hydrops. P_{O_2} and P_{CO_2} and pH changes also were not significant. Normal P_{O_2} in our laboratory, which is 4000 feet above sea level, was 23.3 ± 2.6 mm Hg. However, a statistically significant change was seen in serum protein and albumin levels. With right atrial pacing at 300 beats/min it was possible to achieve hydrops between 18 and 41 hours (Table II). Spontaneous resolution of hydrops occurred in each lamb after systolic-diastolic pacing was discontinued.

The changes in mean systolic/diastolic ratio from baseline in the descending thoracic aorta and umbilical artery for the six lambs are summarized in Fig. 1. There was a significant decrease in systolic/diastolic ratio from baseline at the time hydrops developed. Both vessels showed a significant increase in systolic/diastolic ratio immediately after pacing was withdrawn with hydrops still present. Even though the data are presented as mean values, the Doppler changes described above were seen in each fetal lamb studied.

Fig. 2 illustrates the individual changes that occurred in mean aortic velocity and volume flow throughout the experiments. The trends were similar to those described for systolic/diastolic ratio. All animals studied demonstrated a fall in both parameters during the development of hydrops by pacing and an immediate rise when the effect of pacing was removed. In ewe No. 4 the fetal position precluded obtaining any quantitative information from the aorta, and in ewe No. 6 volume flow information is not presented, because it was unobtainable at several of the time intervals studied.

Table III summarizes the changes observed in fetal blood pressure during the study. There was a statistically significant decrease in systolic blood pressure and an increase in venous pressure with the development of hydrops. Both systolic and venous pressures returned to the prepacing levels during the recovery phase.

The normal flow velocity waveform of the inferior vena cava is biphasic, with the larger peak indicative of the systolic-to-diastolic surge[6] (Fig. 3). This relationship is disturbed during pacing in that (1) the biphasic pattern no longer exists and (2) reverse flow in the inferior vena cava is an early change.

After pacing is discontinued the systolic and diastolic surges reappear, reverse flow is no longer apparent, and by 6 hours the normal relationship of the systolic-to-diastolic surge is restored.

Comment

The atrial tachycardia model for the investigation of fetal hydrops was first described by Stevens et al.[5] Ultrasound imaging was not utilized in these experiments, and the presence of hydrops had to be confirmed at autopsy. The improvements in this study include (1) the tachycardia was directly visualized in order to ensure that it was continuous, (2) the time taken for the production and clearance of hydrops was more precisely defined, (3) Doppler blood flow evaluation could

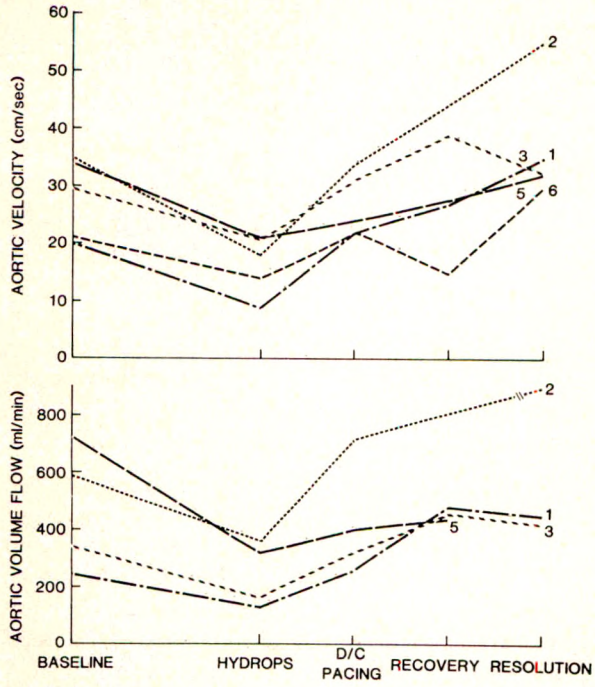


Fig. 2. Changes in the mean aortic velocity and volume flow in individual animals under study.

Table III. Mean fetal blood pressure (mm Hg) (n = 6)

Blood pressure	*Baseline*	*Hydrops*	*Resolution*
Systolic	75.8 ± 6.0	67.1 ± 7.9*	73.0 ± 4.6
Diastolic	53.8 ± 4.8	51.6 ± 3.6	52.8 ± 2.0
Jugular	24.6 ± 5.3	31.3 ± 7.4†	24.6 ± 4.3

*$p < 0.01$.
†$p < 0.06$.

be performed, and (4) the need for prolonged pacing, possibly leading to death, was eliminated.

The pathogenesis of fetal hydrops is not known, but it is postulated that venous hypertension leads to loss of albumin into the extravascular space, with the resultant decreased osmotic and increased venous pressure combining to produce hydrops.[3] Our results agree with this postulate. Because of the obvious difficulty of continuous monitoring of the sheep, our study does not answer the question of the natural progression of changes seen during the production of hydrops. However, ascites was seen first and then became more severe along with the development of pleural effusions and abdominal wall edema. Our studies were performed with right atrial pacing only; it has previously been noted in animal studies that pacing of an atrium results in a decrease in the ipsilateral ventricular output. Therefore, with right atrial pacing a decrease in flow

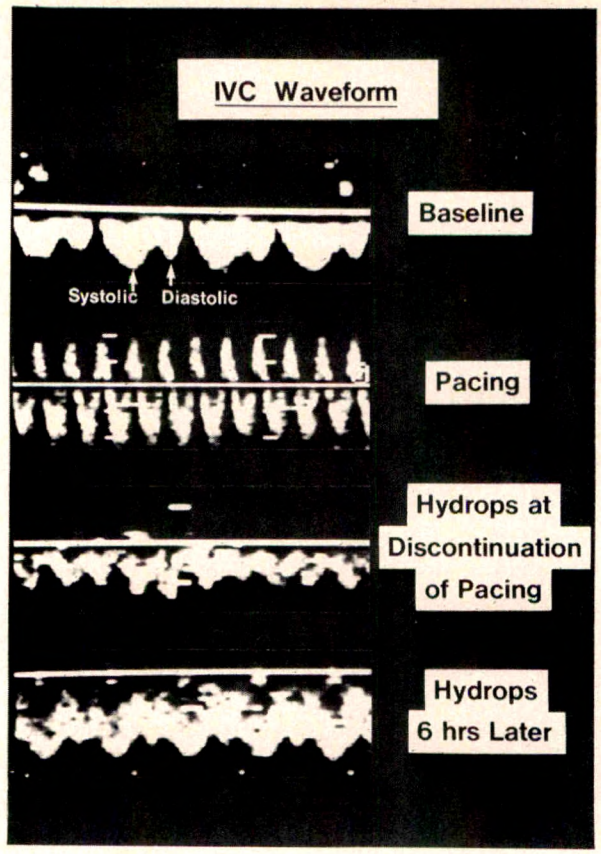


Fig. 3. The inferior vena cava *(IVC)* waveform under different experimental conditions.

in the descending thoracic aorta would be anticipated; this in fact was our observation (Fig. 2). When pacing was discontinued a prompt and significant increase in velocity and volume flow occurred. The immediate improvement after pacing would support the fact that the fetal myocardium was not dysfunctional, although our study did not specifically evaluate myocardial ischemia and function. Other investigators working with an isoproterenol-induced tachycardia have shown the preservation of intramyocardial blood flow during pacing but a decrease in myocardial glycogen.[7] The Doppler changes seen at the time of fetal hydrops are a summation of changes caused by tachycardia and those secondary to hydrops. In a limited number of animals (four) umbilical systolic/diastolic ratio was recorded at 16 hours after pacing was initiated, but before hydrops developed. In each of these the umbilical systolic/diastolic ratio was significantly lower than baseline values. In all animals the systolic/diastolic ratio found immediately on discontinuation of pacing with hydrops present was also significantly different from baseline values.

These Doppler studies add new information on changes in venous and arterial flow patterns in the hydropic animal model. The changes noted in the pressure and flow in the inferior vena cava concur with the

observations of Reuss et al.[8] A trend in our data suggests that an inverse correlation exists between the baseline venous pressure and the time to development of hydrops. Statistical significance was, however, not achieved. During the recovery phase after pacing the normal biphasic pattern of flow in the inferior vena cava with a predominant systolic peak, was rapidly established.

The Doppler waveform in the descending aorta and umbilical artery showed a significant decrease in systolic/diastolic ratio in all animals during hydrops. This was complemented by a decrease in both volume flow and velocity in the aorta. Prompt increases in all of these measurements after pacing was terminated are the observed features of the recovery phase of hydrops.

The design of our study differed from that of Stevens et al.[5] in that their period of pacing was much longer (72 to 216 hours) and several of their animals (six) died during pacing. It is not surprising therefore that even though the baseline levels of PaO_2 were low but similar in both studies, a greater decrease was seen after pacing by Stevens et al. We recognize that the baseline PaO_2 levels were lower than expected; however, none of our animals died during the experimental or recovery period or were otherwise compromised.

Our studies also demonstrated decreases in serum protein and albumin at the time of development of hydrops and return to baseline levels in the recovery period. These changes were not seen by Stevens et al., and one can only speculate that this was probably due to the more chronic nature of their preparation.

In summary, in this study of an animal model of fetal hydrops produced by rapid atrial pacing, we have observed venous hypertension, a drop in serum protein, and an alteration in the venous flow pattern but no significant change in fetal blood gases. The aortic velocity and volume flow during tachycardia-induced hydrops were decreased but showed a prompt increase after pacing was terminated; this increase continued during the recovery phase. The systolic/diastolic ratio was consistently decreased during pacing and at development of hydrops and also was observed to rise again during the recovery period. The Doppler analysis of flow in the fetal vessels has provided new and important information on the fetal changes during tachycardia-induced hydrops and fetal recovery.

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Digoxin-like immunoreactive substance in pregnancy

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Our aims were to determine the potential usefulness of digoxin-like immunoreactive substances in the prediction of preeclampsia, to study the relationship between fetal production of these substances and maternal serum levels, and to evaluate the association between digoxin-like immunoreactive substances and plasma volume findings in preeclamptic pregnancies. Serum digoxin-like immunoreactive substance concentrations were measured in normotensive and preeclamptic pregnant women and in umbilical artery and vein blood samples. None of the patients in the first trimester (n = 53) and 11% of those in the second (n = 56) had detectable levels of this substance. However, 91% of the patients in the third trimester (n = 161) had positive results. The concentrations of digoxin-like immunoreactive substances in the preeclamptic group (n = 78) were significantly ($p < 0.005$) lower than those of third-trimester (n = 83) normotensive patients (0.22 ± 0.12 versus 0.32 ± 0.15 ng/ml). However, there were no significant differences between the two groups regarding digoxin-like immunoreactive substance concentrations when matched for gestational age (41 patients in each group). Digoxin-like immunoreactive substance concentrations in umbilical vessels were significantly higher ($p < 0.001$) than the corresponding maternal levels. Umbilical vessel digoxin-like immunoreactive substance levels demonstrated good correlation with fetal gestational age and birth weight in both normotensive and preeclamptic pregnancies. On the other hand, there was a poor ($r = 0.02$; $p = 0.91$) correlation between plasma volume findings and digoxin-like immunoreactive substance concentration. We conclude that the digoxin-like immunoreactive substance level may be of very little value in the prediction of preeclampsia. The presence of digoxin-like immunoreactive substance at greater concentrations in the umbilical cord blood samples suggests the possibility of the fetus as the source of this substance. Digoxin-like immunoreactive substances may not play a major role in plasma volume expansion during pregnancy. (Am J Obstet Gynecol 1987;157:660-4.)

Key words: Pregnancy, preeclampsia, digoxin-like immunoreactive substance

Endogenous digoxin-like immunoreactive substances that cross react with commercially available digoxin assays have been described by several authors.[1] The presence of this substance has led to falsely elevated digoxin concentrations in patients treated with this drug, as well as measurable levels in others who have never received digitalis glycosides. In addition, digoxin-like immunoreactive substances have been found in varying degrees in persons who suffer from chronic renal failure, hepatic disease, and chronic hypertension.[1, 2] These substances have also been detected in experimental animals submitted to acute volume expansion and salt loading.[3, 4] Furthermore, digoxin-like immunoreactive substances have been found to be present in varying concentrations during pregnancy,[1, 5-7] in amniotic fluid,[1, 8, 9] and in fetal and neonatal serum.[9-11] Some studies have suggested that the concentration of digoxin-like immunoreactive substances in the serum of preeclamptic patients is significantly higher than that in normotensive pregnant patients.[5-7, 9, 12, 13] To date, the exact structures of these substances have not been clearly defined, but research has been conducted in terms of their physiologic activity. Digoxin-like immunoreactive substances are natriuretic and produce vasoconstriction.[4] They also inhibit ionized sodium/ionized potassium adenosine triphosphatase activity.[10]

The aims of this study were threefold: (1) to determine the potential usefulness of digoxin-like immunoreactive substances in the prediction of preeclampsia; (2) to study the relationship between fetal and maternal concentrations of these substances; and (3) to evaluate the association between digoxin-like immunoreactive substances and plasma volume findings in preeclamptic pregnancies.

Material and methods

Blood samples were collected from normotensive pregnant women in the first (n = 53), second (n = 56), and third (n = 83) trimesters. None of the patients suffered from chronic hypertension and none had

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Table I. Concentrations of digoxin-like immunoreactive substances by trimester

Trimester	*Concentration (ng/ml)*		*% Positive*
	Mean ± SD	*Range*	
First (n = 53)	0	0	0
Second (n = 56)	0.02 ± 0.07	0-0.47	11
Third (n = 161)	0.27 ± 0.15	0-0.93	91
Preeclamptic (n = 78)	0.22 ± 0.12	0-0.58	86
Normotensive (n = 83)	0.32 ± 0.15	0-0.93	95

taken digoxin before the study. Specimens were also obtained from women in the third trimester (n = 78) who met standard criteria for the diagnosis of preeclampsia.[14] In a subgroup of 25 of these patients plasma volume was determined simultaneously with digoxin-like immunoreactive substance measurements. Plasma volume measurements were done according to the Evans blue dye-dilution technique as described previously.[15] At the time of delivery, samples were drawn from the umbilical artery, umbilical vein, and maternal vein (n = 25). Specimens were centrifuged immediately, and the serum was stored at −70° C until analysis (within 30 days of collection). Samples were analyzed in duplicate by the RIANEN digoxin assay (New England Nuclear, North Billerica, Massachusetts). The lower limit of sensitivity of the assay is 0.11 ng/ml, with a coefficient of variation of 6.1% at a concentration of 0.5 ng/ml and 3.6% at 2.5 ng/ml. All values below the lower limit of sensitivity of the assay were considered zero in analysis of data.

Neonatal outcome included gestational age and birth weight. Unless otherwise noted, all data are reported as mean and SD. Data were analyzed by regression analysis and paired and unpaired Student's *t* test. Significance was set at $p < 0.05$.

Results

A total of 270 patients were included in this study. Two hundred forty-five patients were studied antepartum and 25 were studied intrapartum. In the antepartum group, two patients had preeclampsia in the second trimester and 78 had it in the third trimester. The average gestational age at time of sampling was 10.2 ± 1.7 weeks in the first trimester group, 18.5 ± 2.6 weeks in the second-trimester group, and 37.7 ± 3.7 weeks in the third-trimester group. Simultaneous samples from mother and umbilical vessels were obtained in 25 patients admitted in labor; six of them had preeclampsia.

Table I summarizes the distribution of digoxin-like immunoreactive substance concentrations by trimester. None of the patients in the first trimester had measurable digoxin-like immunoreactive substance levels. Only six (11%) of those in the second trimester had detectable levels; one of these developed preeclampsia in the third trimester, while the other five remained normotensive. In contrast, 146 (91%) of third-trimester tests yielded positive results. The mean digoxin-like immunoreactive substance concentrations in third-trimester women with preeclampsia was 0.22 ± 0.12 ng/ml, significantly lower ($p < 0.005$) than the value (0.32 ± 0.15 ng/ml) in normotensive third-trimester patients. However, there was a considerable overlap in concentrations in the two groups.

As can be expected, patients with preeclampsia had significantly higher systolic (145 ± 16.2 versus 121 ± 13.5 mm Hg, $p < 0.0001$) and diastolic (96 ± 13.1 versus 73 ± 11.3 mm Hg; $p < 0.0001$) blood pressures, were delivered earlier in gestation (36.5 ± 3.8 versus 38.9 ± 2.7 weeks; $p < 0.001$), and had lower birth weight babies (2634 ± 684 versus 3069 ± 474 gm; $p < 0.028$). In addition, there were strong correlations between digoxin-like immunoreactive substance concentrations and fetal gestational age ($r = 0.98$; $p < 0.0004$) and birth weight ($r = 0.53$; $p < 0.03$) in normotensive patients. Similar results were observed in the group of women with preeclampsia ($r = 0.73$ [$p = 0.005$] and $r = 0.50$ [$p = 0.01$], respectively).

To correct for differences in gestational age between the two groups, we compared the digoxin-like immunoreactive substance findings in 41 patients matched by gestational age with a similar number of normotensive patients. We found no significant differences in digoxin-like immunoreactive substance concentrations between the two groups.

The distribution of digoxin-like immunoreactive substance values in the mother, umbilical vein, and artery are shown in Fig. 1 as Tukey box plots. The mean digoxin-like immunoreactive substance concentration in cord arterial blood was significantly higher than the mean maternal concentration (0.83 ± 0.46 versus 0.29 ± 0.24 ng/ml; $p < 0.001$). Similar findings were observed between cord venous blood and maternal concentrations (0.79 ± 0.41 versus 0.29 ± 0.24 ng/ml; $p < 0.001$). However, there were no significant differences between cord arterial and venous cord blood

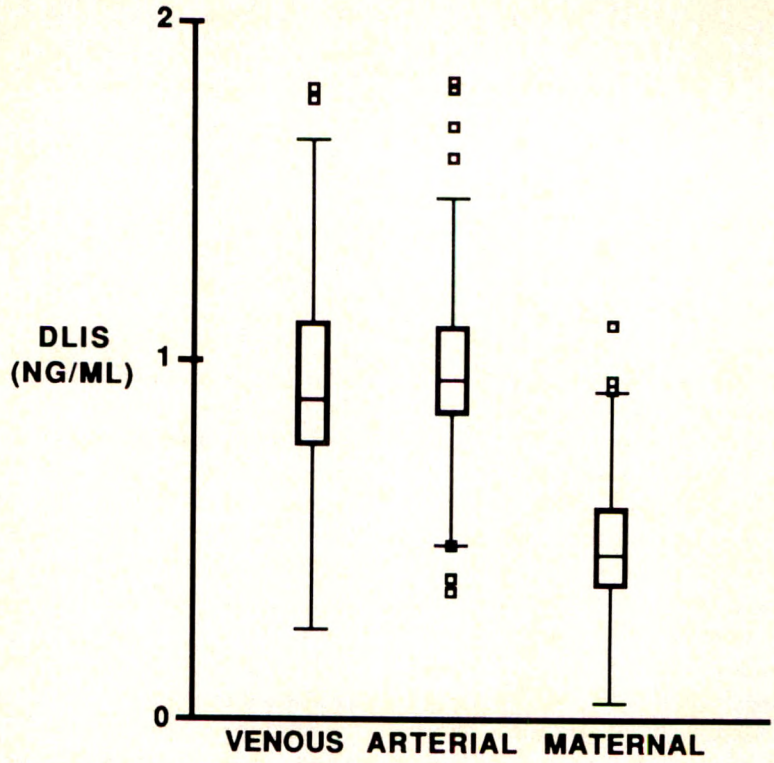


Fig. 1. Umbilical artery, umbilical vein, and maternal digoxin-like immunoreactive substance concentrations *(DLIS)* at the time of delivery. □ Represents valves outside ± SD; □□ indicates median and distribution of 50% of the valves; I shows ±2 SD.

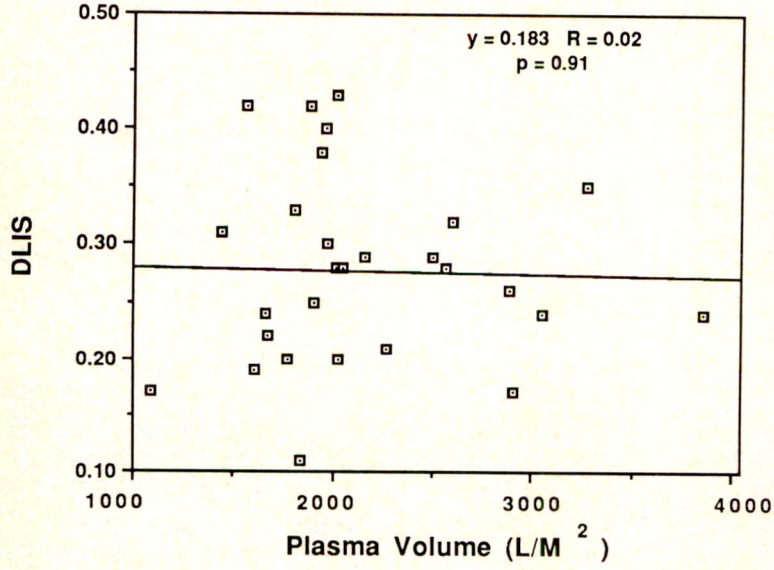


Fig. 2. Correlation between plasma volume findings and digoxin-like immunoreactive substance concentrations *(DLIS)* in preeclamptic pregnancies.

digoxin-like immunoreactive substance concentrations. Moreover, there were good correlations between cord blood digoxin-like immunoreactive substance values and both fetal gestational age ($r = 0.53$; $p < 0.01$) and birth weight ($r = 0.69$; $p < 0.03$).

Fig. 2 correlates digoxin-like immunoreactive substance concentrations to plasma volume findings in the 25 patients studied. It is obvious that there is a poor correlation between these substances and the plasma volume.

Comment

There are several reports describing digoxin-like immunoreactive substance findings during pregnancy. Most previous studies have evaluated serum digoxin-like immunoreactive substance levels during the third trimester.[5, 7] However, Valdes et al.[5] studied digoxin-like immunoreactive substance concentrations in normal pregnancy during the first (n = 11), second (n = 17), and third (n = 20) trimesters. The mean (±SEM) digoxin-like immunoreactive substance concentrations were 0.045 ± 0.01, 0.125 ± 0.03, and 0.195 ± 0.03 ng/ml, respectively. It is apparent from these findings that very few patients had detectable digoxin-like immunoreactive substance concentrations during the first and second trimesters. Our findings are similar to those reported by Valdes et al.

The pathophysiologic mechanisms responsible for the development of preeclampsia are not well understood. Because the physiologic activities of digoxin-like immunoreactive substances include natriuresis, ionized sodium/ionized potassium adenosine triphosphatase inhibition, and peripheral vasoconstriction, some authors have proposed that digoxin-like immunoreactive substance may have an etiologic role in the pathogenesis of preeclampsia. Valdes et al.[5] compared digoxin-like immunoreactive substance concentrations in 22 patients with hypertension in the third trimester with those found in 20 normotensive pregnancies. They found significantly higher values in the hypertensive group. Although their normotensive and hypertensive groups had comparable gestational ages, it is not clear if the patients were matched for gestational age and whether any of the patients with hypertension were chronically hypertensive. Gusdon et al.[7] compared digoxin-like immunoreactive substance concentrations in 10 third-trimester preeclamptic pregnancies with those in 10 normotensive pregnancies in the third trimester. They reported significantly higher values in the preeclamptic group. They speculated that "the increase in blood pressure in preeclampsia is somehow related to increased levels of the natriuretic hormone." It is interesting to note that the average digoxin-like immunoreactive substance concentration (0.14 ± 0.15 ng/ml) in the preeclamptic group in their study was actually lower than the average (0.195 ± 0.03 ng/ml) in the normotensive group described by Valdes et al.

Beyers et al.[6] studied digoxin-like immunoreactive substance concentrations in 36 normotensive and 21 preeclamptic patients at an average gestational age of 38.3 weeks. The average birth weight in both groups was similar, while the average digoxin-like immunoreactive substance concentration was higher in the preeclamptic group (0.68 ± 0.3 versus 0.52 ± 0.06 ng/ml). However, these authors considered all values <0.5 ng/ml to be 0.5 ng/ml, and seven of the preeclamptic patients had preexisting chronic hypertension. More recently, Goodlin[13] reported on 59 patients with expanded toxemia syndrome. Digoxin-like immunoreactive substance levels were measured in 27 patients, with 67% of them having abnormal values (>0.2 ng/ml).

Secombe et al.[9] determined digoxin-like immunoreactive substance concentrations in 36 amniotic fluid samples obtained between 16 and 43 weeks' gestation. They found that the concentration in amniotic fluid was constant from 16 to 33 weeks' gestation but increased after 33 weeks. In the same report, the authors compared digoxin-like immunoreactive substance concentrations in umbilical artery, umbilical vein, and maternal serum samples. There was no difference between umbilical vein and artery concentrations, but umbilical vein concentrations were consistently higher than those in the mother. Their findings suggested that digoxin-like immunoreactive substance was of fetal origin. In a similar report Beyers et al.[6] found mean digoxin-like immunoreactive substance levels to be higher in cord blood than in maternal blood. Our findings regarding cord blood and maternal digoxin-like immunoreactive substance concentrations are in agreement with both of these reports. In addition, we found good correlations between digoxin-like immunoreactive substance values and both fetal gestational age and birth weight. Hence a fetal origin for this substance seems very likely.

We found lower levels of digoxin-like immunoreactive substance in the preeclamptic group than in the normotensive group. However, the preeclamptic group had both lower gestational ages and birth weights. When the two groups were matched for gestational age, we found no differences in digoxin-like immunoreactive substance concentrations between preeclamptic and normotensive pregnancies. In addition, there was considerable overlap in concentrations in the two groups. These findings are in disagreement with those reported previously. The differences in our data as compared with those of others[6, 7, 9, 12, 13] may be due to differences in populations studied, differences in gestational ages, and different methods of extraction of the substance and the assays used. The radioimmunoassay we used (RIANEN) is the same assay used by Valdes et al.,[5] Graves et al.,[12] and as Gusdon et al.[7] However, Gusdon et al. and Beyers et al.[6] used an extraction procedure. Moreover, the radioimmunoassay technique used by Beyers et al.[6] is similar to our technique. However, these investigators considered all values <0.5 ng/ml as an absolute value of 0.5 ng/ml, and seven of the patients with preeclampsia had preexisting chronic hypertension. These factors may account for the observed differences. The reliability of the radioimmunoassay technique in pregnancy has been recently validated by Clerico et al.,[16] who found a significant

correlation between digoxin-like immunoreactive substance values obtained by radioimmunoassay methods and radioreceptor assays for 21 chromatographic extracts of plasma from pregnant women and neonates. These authors stated that the digoxin radioimmunoassay method can be used for digoxin-like immunoreactive substance determinations because it is "more sensitive, precise, and simple than other techniques used to detect endogenous cardiac-glycosides–like compounds." The fluorescence polarization immunoassay used by Goodlin[13] is less specific for digoxin-like immunoreactive substance because it shows cross-reactivity with progesterone, cortisol, and cortisone. The use of this technique could have influenced the results of that study.

Because digoxin-like immunoreactive substances are rarely detected in the first and second trimesters and the concentrations are not elevated in preeclampsia in the third trimester, it is very unlikely that this test will be helpful in predicting preeclampsia.

In normal pregnancy, plasma volume expansion starts very early in the first trimester, reaches a plateau at 24 to 34 weeks' gestation, and is maintained until term.[15] We found a poor correlation between digoxin-like immunoreactive substance concentrations and plasma volume in the preeclamptic subgroup. Additionally, digoxin-like immunoreactive substance was not detected in the first trimester and only 11% of patients had measurable levels in the second trimester. These findings suggest that digoxin-like immunoreactive substance does not play any major role in plasma volume expansion during pregnancy.

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Now

The next generation of estrogen replacement therapy...

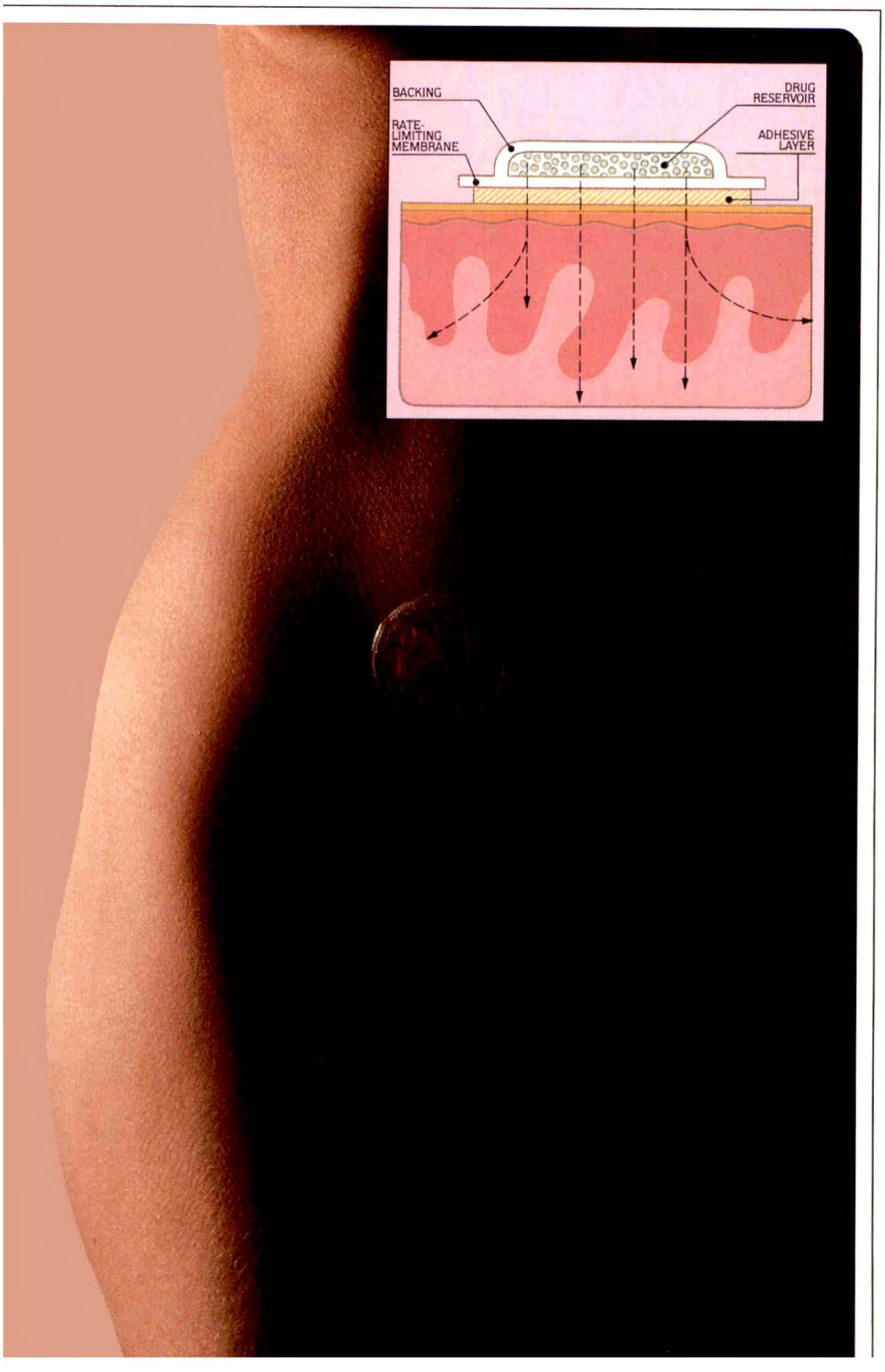
transdermal delivery



ESTRADERM®

estradiol transdermal syster

Combines the advantages of transdermal delivery...



A specially designed transdermal delivery system

Providing the natural ovarian estroge (17-beta estradiol) directly to the bloodstream and target organs; with no first-pass liver metabolism, permi use of extremely low dosage

Effectively controls estrogen deficiency symptoms

Steady-state relief of hot flashes, oth menopausal symptoms associated wi hot flashes, and vaginal discomfort

Well tolerated

Very low incidence of systemic side effects...few local problems of consequence

Easy to use; transdermal delivery preferred 3:1 by patients over tablets[1]

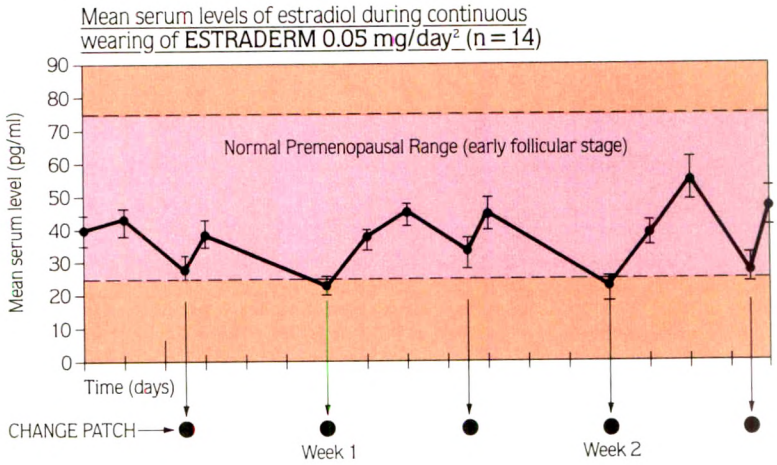
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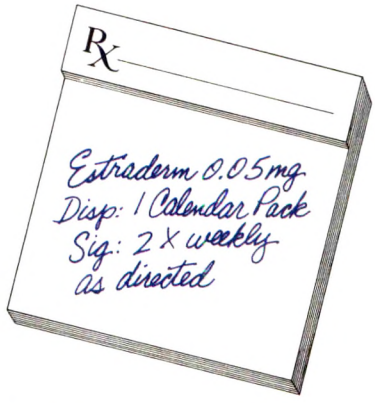
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le in dose-reminder calendar packs of eight
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I B A
g today's women live healthier lives

References:

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Please see next page
for Brief Summary of Prescribing Information.

ESTRADERM®

estradiol transdermal system

Continuous delivery for twice-weekly application.
BRIEF SUMMARY OF PRESCRIBING INFORMATION.
PLEASE SEE FULL PRESCRIBING INFORMATION.

ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than 1 year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer-reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5-13.9 times greater than in nonusers. The risk appears to depend both on duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that women who had been exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than four per 1000 exposures. Furthermore, a high percentage of such exposed women (30-90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data on the use of other estrogens are not available, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7-fold increased risk of limb-reduction defects in infants who had been exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estraderm is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and of the advisability of continuation of the pregnancy.

INDICATIONS AND USAGE

Estraderm is indicated for the treatment of the following: moderate-to-severe vasomotor symptoms associated with menopause; female hypogonadism; female castration; primary ovarian failure; and atrophic conditions caused by deficient endogenous estrogen production, such as atrophic vaginitis and kraurosis vulvae.

Studies of the addition of a progestin for seven or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of endometrium suggest that 12 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks that may be associated with the inclusion of progestin in estrogen replacement regimens. The potential risks include adverse effects on carbohydrate and lipid metabolism. The choice of progestin and dosage may be important in minimizing these adverse effects.

CONTRAINDICATIONS

Estrogens should not be used in women or men with any of the following conditions:

1. known or suspected cancer of the breast;
2. known or suspected estrogen-dependent neoplasia;
3. known or suspected pregnancy (see Boxed Warning);
4. undiagnosed abnormal genital bleeding;
5. active thrombophlebitis or thromboembolic disorders;
6. history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. Induction of Malignant Neoplasms. Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time, there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of breast cancer, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. Gallbladder Disease. A recent study has reported a two- to threefold increase in the risk of surgically confirmed gallbladder disease in postmenopausal women receiving oral estrogens, similar to the twofold increase previously noted in users of oral contraceptives.

3. Effects Similar to Those Caused by Estrogen-Progestogen Oral Contraceptives. There are several serious adverse effects of oral contraceptives and other high-dose oral estrogen treatments, most of which have not, up to now, been documented as consequences of postmenopausal estrogen replacement therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women.

a. Thromboembolic Disease. It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least four weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. Hepatic Adenoma. Benign hepatic adenomas have been associated with the use of oral contraceptives. Although benign and rare, these tumors may rupture and cause death from intra-abdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations, but they should be considered if abdominal pain and tenderness, abdominal mass, or hypovolemic shock occurs in patients receiving estrogen. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The causal relationship of this malignancy to these drugs is not known.

c. Elevated Blood Pressure. Women using oral contraceptives sometimes experience increased blood pressure which, in most cases, returns to normal upon discontinuing the drug. There is now a report that this may occur with use of oral estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used. Ethinyl estradiol and conjugated estrogens have been shown to increase renin substrate. In contrast to these oral estrogens, transdermally administered estradiol does not affect renin substrate.

d. Glucose Tolerance. A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. Hypercalcemia. Administration of high doses of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If hypercalcemia occurs, use of the drug should be stopped and appropriate measures should be taken to reduce the serum calcium level.

PRECAUTIONS

General

1. A complete medical and family history should be taken before initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, as well as a cervical Papanicolaou test. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.
2. Because estrogens may cause some degree of fluid retention, careful observation is required when conditions that might be influenced by this factor are present (e.g., asthma, epilepsy, migraine, and cardiac or renal dysfunction).
3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as uterine bleeding, mastodynia, etc.
4. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Estrogens should be used with caution in patients who have or have had endometriosis.
5. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.
6. Preexisting uterine leiomyomata may increase in size during prolonged estrogen use. If this occurs, estrogen therapy should be discontinued while the cause is investigated.
7. In patients with a history of jaundice during pregnancy, there is an increased risk that jaundice will recur with the use of estrogen-containing oral contraceptives. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.
8. Estrogens may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.
9. Because the prolonged use of estrogens influences the metabolism of calcium and phosphorus, estrogens should be used with caution in patients with metabolic bone diseases associated with hypercalcemia and in patients with renal insufficiency.

Drug/Laboratory Test Interactions

The results of certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following changes have been observed with large doses of oral estrogen:

1. increased sulfobromophthalein retention;
2. increased prothrombin time; increased factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability;
3. increased thyroxine-binding globulin (TBG), leading to increased circulating total thyroid hormone (T_4) as measured by column or radioimmunoassay; free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered; TBG was not affected in clinical trials of Estraderm;
4. reduced response to the metyrapone test;
5. reduced serum folate concentration;
6. increased serum triglyceride and phospholipid concentration, and decreased pregnanediol excretion.

The pathologist should be informed that the patient is receiving estrogen therapy when relevant specimens are submitted.

Carcinogenesis, Mutagenesis, Impairment of Fertility

See **WARNINGS** and Boxed Warning.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

Pregnancy Category X

See **CONTRAINDICATIONS** and Boxed Warning.

Estrogens should not be used during pregnancy.

Nursing Mothers

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

See **WARNINGS** and Boxed Warning regarding potential adverse effects on the fetus, induction of malignant neoplasms, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.

The most commonly reported adverse reaction to Estraderm in clinical trials was redness and irritation at the application site. This occurred in about 17% of the women treated and caused approximately 2% to discontinue therapy. Reports of rash have been rare.

The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

Genitourinary System: Breakthrough bleeding, spotting, change in menstrual flow; increase in size of uterine fibromyomata; change in cervical erosion and amount of cervical secretion.

Endocrine: Breast tenderness, breast enlargement.

Gastrointestinal: Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice have been observed with oral estrogen therapy.

Eyes: Steepening of corneal curvature; intolerance to contact lenses.

Central Nervous System: Headache, migraine, dizziness.

Miscellaneous: Change in weight, edema, change in libido.

Do not store above 86°F (30°C).

Do not store unpouched. Apply immediately upon removal from the protective pouch.

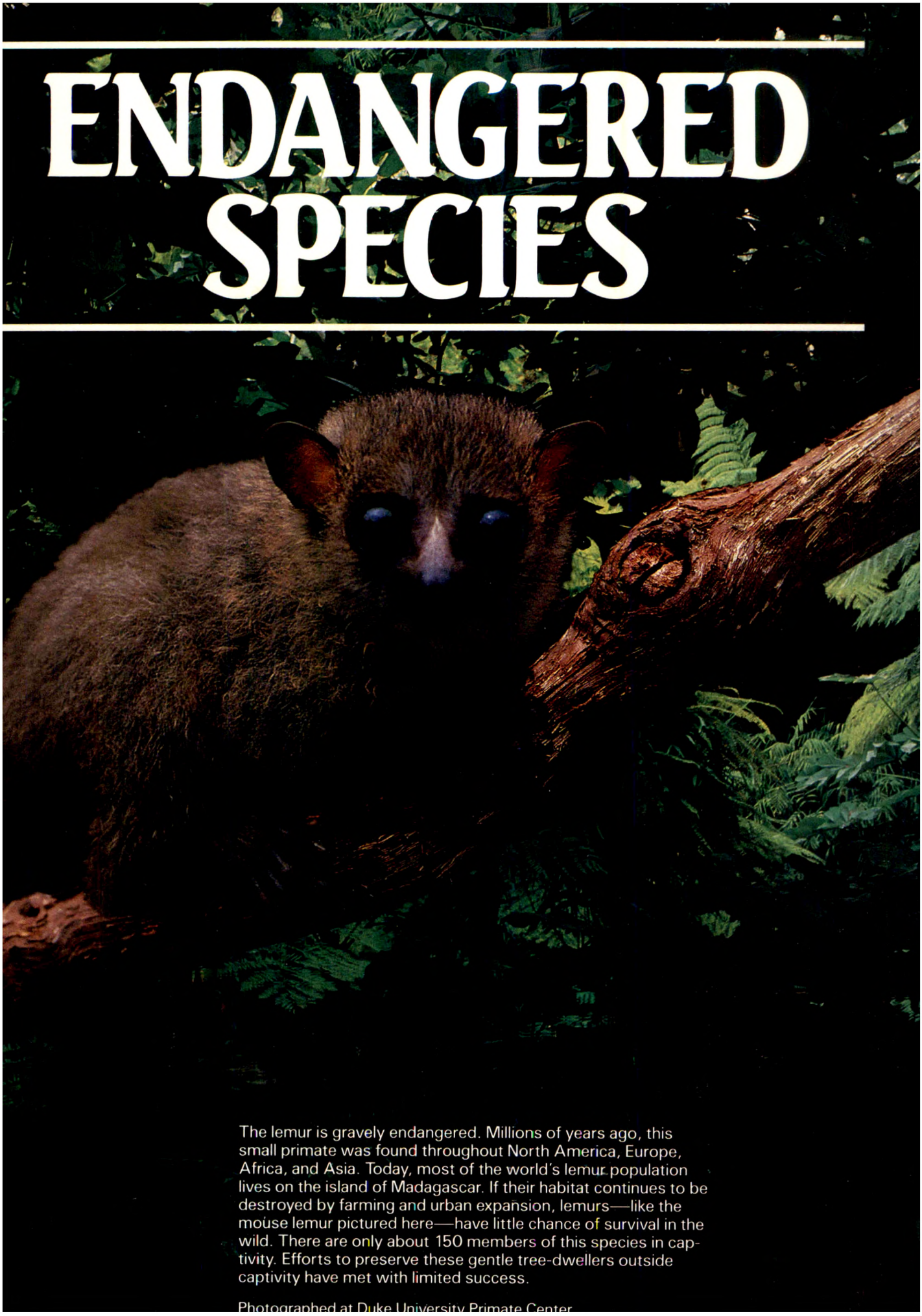
CAUTION: Federal law prohibits dispensing without prescription.

C I B A

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ENDANGERED SPECIES



The lemur is gravely endangered. Millions of years ago, this small primate was found throughout North America, Europe, Africa, and Asia. Today, most of the world's lemur population lives on the island of Madagascar. If their habitat continues to be destroyed by farming and urban expansion, lemurs—like the mouse lemur pictured here—have little chance of survival in the wild. There are only about 150 members of this species in captivity. Efforts to preserve these gentle tree-dwellers outside captivity have met with limited success.

Photographed at Duke University Primate Center

ENDANGERED SPECIES?

Is the practice of obstetrics endangered? Its survival is threatened by factors that have the potential to diminish significantly the number of practicing obstetricians. The continuing increase in the number of malpractice lawsuits against physicians and the corresponding inflation of liability insurance premiums have plunged the obstetrics specialty into a crisis.

Patient-initiated litigation is now so common that 1 in 6 obstetrician/gynecologists is sued each year, compared with 1 in 20 only ten years ago. As a result, obstetricians pay three to four times more for malpractice insurance than do physicians in most other specialties.

Survival of the practice of obstetrics is further jeopardized because fewer students are entering the specialty and the number of experienced physicians leaving obstetrics or retiring early is on the rise. For many physicians, the crisis has taken the joy out of practicing medicine.

AVOIDING THE DANGER OF EXTINCTION

Immediate action is necessary in order to preserve the practice of obstetrics. In response to this need, G.D. Searle & Co. has organized the Searle Alliance for Health Care. Its purpose is to help lead a national effort to resolve the medical professional liability crisis.

Components of a campaign developed by the Alliance include: nationwide teleconferences and audioconferences; strategy to petition legislators for reform; educational programs and seminars; and national advertising to raise public consciousness of the crisis.

Operating on a national and personal level, the Searle Alliance for Health Care can provide information to help you in your practice. Learn more about medical professional liability and how we can provide information about the problem of professional liability. Contact your Searle representative for more information on the Searle Alliance for Health Care.

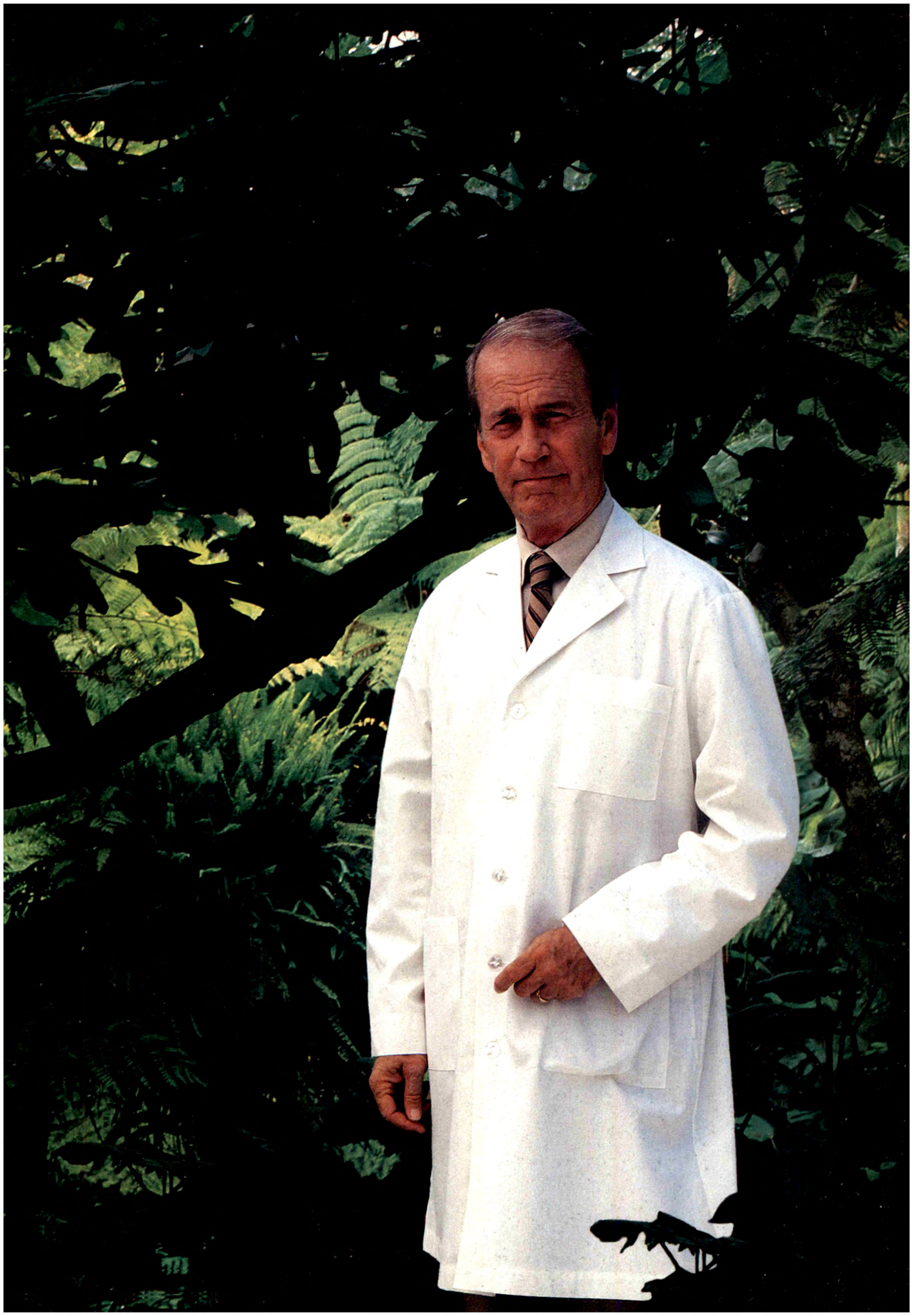


SEARLE
Seeking resolution of the professional liability crisis

Please see the last page of this advertisement for a brief summary of the complete prescribing information.

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Demulen 1/35™
(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)



Demulen 1/35™

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Demulen 1/35™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients.

Demulen 1/50™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients. **Indication and usage:** For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk:* Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen. **Contraindications:** Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products. **Warnings:**

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age: <30	30-39	≥40
Heavy smokers (≥15/day)	C	B	A
Light smokers (<15/day)	D	C	B
Nonsmokers, no predispositions	D	C,D	C
Nonsmokers, with predispositions	C	C,B	B,A

Be alert to earliest manifestations of TE and thrombotic disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. The RCGP prospective study reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. The data suggest varicose veins have little effect on development of deep vein thrombosis but substantially increase risk of superficial vein thrombosis, risk depending on severity of varicosities. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is evidence that estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions (eg, irregular bleeding before OCs, polycystic ovaries) occur nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer v combination or progestogen-only OCs, though individual cases were reported. Studies show no increased breast cancer with OCs or estroge However, greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk of bre cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens rose and further increa with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other risk factors. Decrease in benign bre tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, erosion, and carcinoma in long-term usi Cervical microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increased car risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consi nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of breast car or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. **4.** *Hepatic adenomas,* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk, as was age o 30. Though benign, these lesions may rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk duration of use, being much greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,0 Consider such lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to half w acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT n be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. OCs are contraindicated in case of past present, benign or malignant liver tumors developing during use of estrogenic products. **5.** *Use in or immediately preceding pregnancy, b defects, malignancy in offspring:* Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in ut to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, moni such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. Di exposed daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Other estrogi may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL for vertebral, anal, cardi tracheoesophageal, renal, and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of lir reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threater abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, includ OCs, during early pregnancy occurred in 18.2/1,000 births, compared to 7.8 for those unexposed (P<.05). More twinning was reported conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but there is considera evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased abortuses from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion increased soon after stopping OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not be demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods a missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. pregnancy is confirmed, apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy sho use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not u progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 yea use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. **7.** *Carbohydrate and lipid metabolism:* Decreas glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides and to phospholipids may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few months, and degree may relate to progestog dose. Hypertension prevalence in users is not higher in the first year, but increases with longer exposure; in the 5th year it is 2½—3 tim the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), preexisting ren disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weig gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Ev though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of migraine development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating cause. **10.** *Bleedi irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregu bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology excluded, time or change to another product may suffice. Change to a higher-estrogen OC only when necessary as this may increa thromboembolic risk. Those with history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatc or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulatic possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has be associated with postpill amenorrhea. One stucy reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients wi postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio of ectopic to intrauteri pregnancies is no higher than in nonusers. There may be increased risk of ectopics after discontinuing. **12.** *Breast feeding:* OCs may decrea quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs un after weaning. **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable differen in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 month and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete histo and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uterir leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if serious recurrence. Flu retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic, or ren dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestat jaundice occurred after OC and troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblast anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use whe submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have bee observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, D X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyroi hormone (PBI, T^4); decreased T^3 uptake, unaltered free T^4, decreased pregnanediol excretion; reduced metyrapone response; increased bloc transcortin, corticosteroid, triglyceride, phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influenc of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask onsi of climacteric. Cervical chlamydial prevalence is increased; do not assume OC protects against chlamydial PID. *Information for patients.* Se patient labeling. *Drug interactions.* OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycir penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidon phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizer tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophen **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis an thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; coronary thrombosis; cerebral thrombo sis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The followin are believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhe during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargemen secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change i corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted for: premenstrual-like syndrome, cataracts libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hemolytic uremia malignant hypertension, itching, vaginitis, porphyria, impaired renal function, acute renal failure, sometimes irreversible, PVCs, abnormal EKG pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythematosus, rheumatoi arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanoma, endometrial, cervical, and breas carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea an withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use an is available on request.**

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Management of the diethylstilbestrol-exposed pregnant patient: A prospective study

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Over a 5-year period we have managed 63 diethylstilbestrol-exposed pregnant patients with a standardized protocol requiring weekly cervical examination and decreased physical activity of the patient. Twenty-six patients (42%) underwent a prophylactic cerclage for a history of second-trimester loss or a hypoplastic cervix on initial clinical examination (group I). Thirty-six patients (58%) were followed expectantly (group II). Sixteen patients (44%) in group II demonstrated cervical change and required an emergency cerclage. Twenty-one patients were managed expectantly with no cerclage. The gestational age at delivery for group I was 37.7 ± 2.80 versus 34.5 ± 6.9 weeks for patients without a cerclage ($p = 0.04$). There were no perinatal deaths if a cerclage was performed, whereas there were five deaths (24%) in the group without cerclage. The five deaths occurred at a mean gestational age of 24.40 ± 4.0 weeks and a mean birth weight of 614.00 ± 441.73 gm. Patients with a hypoplastic cervix or prior reproductive loss had a better outcome with early cerclage than patients with a normal cervix followed expectantly. We presently lack a reliable method to detect the diethylstilbestrol-exposed patient at greatest risk for perinatal loss. Based on our experience we believe that placement of a cerclage early in pregnancy should be a strong consideration. (Am J Obstet Gynecol 1987;157:665-9.)

Key words: Cerclage, incompetent cervix, diethylstilbestrol pregnancy

Poor reproductive outcome has been described in several studies of diethylstilbestrol-exposed women exhibiting cervicovaginal abnormalities.[1-3] Adverse pregnancy outcomes have been observed in as many as 58% of these women.[4] Increased incidences of ectopic pregnancy, prematurity, and perinatal death have contributed to pregnancy wastage in diethylstilbestrol-exposed women. Although the frequency of viable pregnancy outcomes has been found to range from 42% to 75%, only 40% to 63.5% of these patients can be expected to reach term.[2-7]

Kaufman et al.[8] have demonstrated a greater incidence of upper genital tract abnormalities in diethylstilbestrol-exposed progeny. When these authors related upper genital tract abnormalities to reproductive outcome, the patients with such abnormalities were found to have worse reproductive outcomes when compared with patients with normal uterine anatomy. These authors, however, could not relate a specific clinical pattern of pregnancy loss to the cervicouterine changes observed. The possibility that cervical incompetence is increased in the diethylstilbestrol-exposed woman was first suggested by Singer and Hochman[9] and was later supported by Goldstein.[10] Successful pregnancy outcomes for these patients with cerclage were subsequently reported.[1] Other authors have cautioned against the liberal use of cerclage in diethylstilbestrol-exposed women, reserving cerclage placement for cases in which the past obstetric history clearly indicates an incompetent cervix.[2, 6, 8]

The purpose of this study was to determine if pregnancy outcome could be improved with a standard management protocol emphasizing the aggressive use of cerclage in diethylstilbestrol-exposed pregnant women. The results of such a study could provide important information for the care of a large group of women who are currently of reproductive age.

Methods

Beginning in 1981, 63 pregnant women with a prior history of diethylstilbestrol exposure in utero received antepartum care in our institution. These women were managed according to a standard protocol of: (1) weekly or biweekly cervical examinations and (2) limited physical activity. Patients who were found on initial examination to have normal cervical length and appearance as well as those without prior pregnancy loss were followed expectantly. Early elective cerclage (12 to 14 weeks) was performed in patients with a hypoplastic cervix (less than 1 cm in length) on initial clinical

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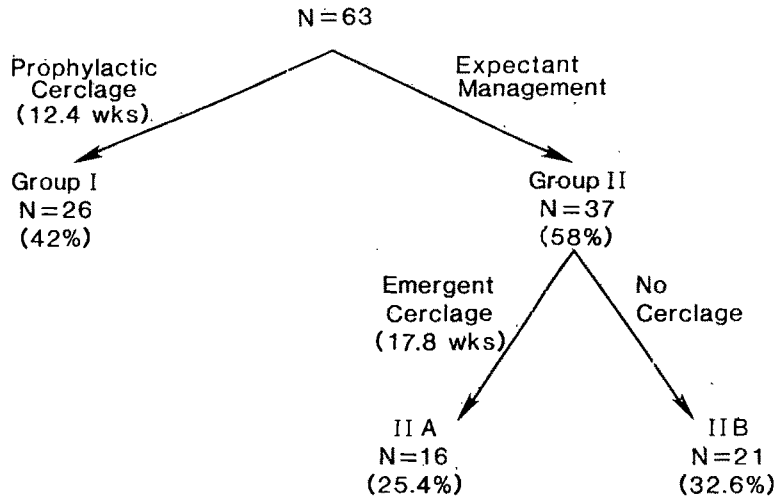


Fig. 1. Management protocol of the 63 diethylstilbestrol-exposed pregnant patients studied.

Table I. Pregnancy outcome in diethylstilbestrol-exposed women undergoing prophylactic (early) cerclage—group I

	Ia; previous loss (n = 13)	*Ib; hypoplastic cervix (n = 13)*
Birth weight (gm)*	2682.92 ± 599.07	3195.46 ± 446.15
Gestational age at delivery (wk)*	36.4 ± 3.1	38.9 ± 1.7
Gestational age at first episode of PML (wk)*	28.2 ± 1.8	27.4 ± 2.9
Days in hospital owing to PML or cervical change*	27.1 ± 19.8	19.7 ± 8.6
PROM	1	1
Perinatal deaths	0	0
Cervical lacerations	1	2

PML, preterm labor; PROM, premature rupture of the membranes.
*There were no significant differences between groups Ia and Ib by Student's *t* test.

examination and in those with a history of prior second-trimester loss. Emergent cerclage was attempted in patients demonstrating cervical effacement or dilatation that was confirmed by two examiners before 22 weeks' gestation. All patients exhibited normal cervical cytology before and during gestation. Weekly cervical examinations were performed after the cerclage was placed. Ultrasound assessment of the lower uterine segment and cervix was not routinely done.

Results

The study group of 63 women consisted of 21 primigravidas, 17 patients in their second pregnancy, and 25 with two or more previous pregnancies. The mean ages for these three groups were 29.9 28.9, and 30.8 years, respectively. Eighteen patients (28.5%) had been studied by hysterosalpingography before pregnancy; 12 of these 18 patients (66%) demonstrated a uterine abnormality. Thirteen patients (21%) were judged to have a hypoplastic cervix. Of the 21 primigravidas six (28.6%) had undergone a previous hysterosalpingography with results being abnormal in three, 24% had a hypoplastic cervix, and in 71.4% examination revealed evidence of cervicovaginal abnormalities, mainly adenosis. For the primigravidas who underwent hysterosalpingography before pregnancy, an abnormal hysterosalpingography predicted only 50% of cases in which premature cervical effacement or dilatation developed.

Of the 63 patients studied 26 (42%) underwent an elective prophylactic cerclage at a mean gestational age of 12.4 weeks (group I). This group consisted of 13 women with a hypoplastic cervix (group Ia) and 13 women with a history of second-trimester loss (group Ib). Thirty-seven patients (58%) were managed expectantly (group II). This group included 16 primigravid women. Sixteen (25.4%) group II patients, designated group IIa, subsequently required emergent cerclage for cervical change at a mean gestational age of 17.8 weeks. The remaining 21 patients, group IIb, were followed expectantly and did not undergo cerclage (Fig. 1).

Table II. Pregnancy outcome in diethylstilbestrol-exposed women managed expectantly—group II

	IIa; emergent cerclage (n = 16)	*IIb; no cerclage (n = 21)*	*p Value*
Birth weight (gm)	2679.69 ± 828.27	2539.04 ± 1219.40	NS
Gestational age at delivery (wk)	36.1 ± 3.3	34.7 ± 6.8	NS
Gestational age at first episode of PML (wk)	24.7 ± 2.4	28.1 ± 4.4	0.05*
Days in hospital owing to PML or cervical change	37.8 ± 25.1	19.9 ± 18.4	
PROM	1	5	NS
Perinatal deaths	0	5	0.06†
Cervical laceration	0	0	NS

PML, preterm labor; PROM, premature rupture of the membranes.
*Student's *t* test.
†Fisher's exact test.

Table III. Comparison of outcome in patients undergoing early elective cerclage versus patients managed expectantly

	Group I; prophylactic cerclage (n = 26)	*Group II; expectant management (n = 37)*		*p Value**
		IIa; emergent cerclage (n = 16)	*IIb; no cerclage (n = 21)*	
Birth weight (gm)	2939.19 ± 579.75	2679.68 ± 828.27	2539.04 ± 1219.40	NS
Gestational age at delivery (wk)	37.7 ± 2.7	36.1 ± 3.3	34.7 ± 6.8	0.04†
PROM	2	2	5	NS
Deliveries <30 wk	1	1	5	NS
Perinatal deaths	0	0	5	0.06‡

PROM, premature rupture of the membranes.
*Comparing group I versus IIb.
†Student's *t* test.
‡Fisher's exact test.

Table I details pregnancy outcome in the elective cerclage group. No perinatal losses were observed in these 26 patients. The only complications encountered were three cervical lacerations repaired without incident at the time of delivery.

Results for those patients managed expectantly (group II) are presented in Table II. Although the birth weights and gestational ages achieved were lower in this group when compared with group I patients, these differences were not statistically significant. There were no perinatal losses observed in the emergent cerclage group, and in only one patient did delivery take place before 30 weeks. Table III compares the outcomes for group I versus groups IIa and IIb. The mean gestational age for the elective cerclage group (37.7 weeks) was significantly greater than that achieved when an elective cerclage was not performed (34.7 weeks, $p = 0.04$). Two patients who underwent cerclage had delivery before 30 weeks. These included a 29-week delivery (birth weight 950 gm) and a 29.5-week delivery (birth weight 1110 gm). Both infants survived. Perinatal losses were significantly higher in patients who did not undergo cerclage. In total 88% of patients who underwent an early prophylactic cerclage were delivered of their infants at term as compared to 75% of the emergency cerclage group and 70% of patients without cerclage. Overall, 54% of the patients in the study experienced preterm labor, and 25% received intravenous tocolytic therapy.

The five perinatal deaths in patients who did not have a cerclage occurred at a mean gestational age of 24.4 ± 4.0 weeks. These infants had a mean birth weight of 614.00 ± 441.73 gm. All of these patients experienced premature rupture of membranes anywhere from 3 to 7 days after a normal cervical examination. Four neonatal deaths were caused by prematurity, and one was due to sepsis. Three of these five patients were primigravid women found to have a normal cervical examination at the time of their initial prenatal visit. The remaining two patients had experienced normal outcomes in prior pregnancies. Our protocol of expectant management failed to prevent perinatal loss in these women. Three patients suffering a perinatal death had previously undergone a hysterosalpingography, and all of them had normal uterine anatomy.

Comment

The great likelihood of adverse pregnancy outcomes previously reported in diethylstilbestrol-exposed women led to the aggressive management of the patients described in this study. In a retrospective study performed at our institution Mangan et al.[11] found an increased incidence of prematurity, premature cervical dilatation, and premature rupture of membranes. Only 75% of diethylstilbestrol-exposed patients had a successful pregnancy versus 89.6% of controls. The primigravid diethylstilbestrol-exposed woman presents the most difficult problem in predicting perinatal outcome. A review of the literature reveals rates of adverse pregnancy outcome as high as 44%, prematurity rates ranging from 15.2% to 28%, and a perinatal death rate between 4% and 6% in these women.[1] These percentages are much higher than expected in the general population. The mean gestational age for primigravid patients in our study was 29.9 years of age, reflecting the time period when diethylstilbestrol was commonly prescribed (1940 to 1971). As some of these women are reaching later reproductive years, a concern is that awaiting recurrent pregnancy loss before identifying their pregnancies as high risk may be inadvisable.

The liberal use of cerclage as part of our protocol for the patient with a history of prior second-trimester loss is generally accepted in the literature, based on studies describing true cervical incompetence in these patients and successful pregnancies after cerclage. There are few prospective double-blind randomized studies concerning the use of cerclage in the nondiethylstilbestrol-exposed population.[12] Most studies are retrospective, and criteria for accurate diagnosis of incompetent cervix are difficult to define.[13] Frequently the diagnosis of incompetent cervix can be made only after a history of painless dilatation in the second trimester. In our study weekly cervical examination failed to predict second-trimester perinatal loss in five of 37 (15%) patients who did not have a prior history of second-trimester loss or hypoplastic cervix. These results emphasize that careful clinical evaluation alone may be inadequate in preventing pregnancy wastage in the diethylstilbestrol-exposed woman.

Ultrasound has been advocated as an objective way of securing the diagnosis of incompetent cervix.[14] However, several artifacts may interfere with sonographic imaging of the cervix in pregnancy.[15] These include bladder distention and angulation of and manual pressure on the transducer. As most studies have included the transabdominal approach, it remains possible that placement of a perineal or vaginal transducer may offer better imaging results.

The etiology of incompetent cervix is poorly understood. Buckingham et al.[16] first described decreased amounts of collagen and a greater percentage of smooth muscle in cervical biopsies obtained from patients with a history of cervical incompetence. To date there are few biochemical or anatomic studies of the cervical stroma in humans. Cousins[17] described a greater percentage of smooth muscle in the cervical stroma of a uterus obtained at hysterectomy in a diethylstilbestrol-exposed patient with a history of incompetent cervix. Laboratory experiments have revealed stromal changes in the endometrium of diethylstilbestrol-exposed mice, with destruction of collagen fibrils. Preliminary observations in our laboratory (J. L.) have demonstrated a decreased amount of collagen and a disarray of collagen fibrils in the cervices of diethylstilbestrol-exposed nonpregnant women.

A hypoplastic cervix cannot be equated with an incompetent cervix; however, a hypoplastic cervix has been associated with an increased risk for adverse pregnancy outcome.[11] For this reason we elected to perform a cerclage in these women. No complications were associated with placement of the cerclage except for three minor cervical lacerations at the time of delivery that were easily repaired. Although we clearly recognize the potential for selection bias in the group of women undergoing cerclage, the finding that most of these patients reached term without perinatal loss strongly suggests a benefit from our management approach. A randomized, controlled study to assess the benefit of cerclage in diethylstilbestrol-exposed primigravid women might seem in order; however, to withhold cerclage placement from those women with obvious abnormal cervical architecture (hypoplastic cervix) does not seem justified in view of our results. A striking finding of this study is that all five perinatal losses occurred in the expectant management group. Within 1 week of a normal cervical examination three primigravidas experienced premature rupture of membranes and perinatal loss. Whether these five losses could have been prevented by early cerclage is uncertain. Until we have established better methods to predict those diethylstilbestrol-exposed pregnant women at greatest risk for second-trimester loss, we believe that an aggressive approach is justified. The excellent results achieved in our elective cerclage group suggest to us that strong consideration be given to placement of a prophylactic cerclage in the diethylstilbestrol-exposed woman, irrespective of cervical appearance or findings on hysterosalpingography.

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Gestational diabetes: Insulin requirements in pregnancy

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A prospective study of 57 women with gestational diabetes mellitus was undertaken to determine actual insulin requirements throughout pregnancy. Women were placed on a multiple injection, mixed insulin regimen and monitored their blood glucose level 6.5 ± 1 times per day using a memory-based reflectance meter to obtain verified data. A significant ($p < 0.01$) increase in total insulin dose was found during the initial treatment period (7 ± 2 days) until the target glucose range was achieved. Insulin requirements continued to significantly ($p < 0.01$) rise until 30 ± 1 gestational weeks, despite a stabilization of glucose level. Thereafter, there was no significant change (3%) in insulin requirement. A correlation of $r = 0.58$ ($p < 0.001$) for the relationship between insulin dose at the 24 and 32 weeks' gestation, and an $r = 0.99$ ($p < 0.0001$) for the relationship between insulin dose at 32 and 39 weeks' gestation was found. We concluded that an emphasis on ambulatory blood glucose control and insulin adjustments should occur in the early treatment phase of gestational diabetes. (AM J OBSTET GYNECOL 1987;157:669-75.)

Key words: Gestational diabetes mellitus, insulin requirements, verified data

The perinatal principle that early stringent and consistent metabolic control decreases fetal mortality and neonatal morbidity in gestational diabetes mellitus is well accepted.[1-4] It has been shown that near normal levels of glycemic control can be achieved through the self-monitoring of blood glucose on an ambulatory basis.[5, 6] Collection of this data allows the accurate anticipation of baseline insulin requirements, as well as any changes in insulin dose necessary to achieve and maintain normoglycemia throughout pregnancy. However, it has been reported that self-monitoring errors occur that may be resolved by the use of memory-based reflectance meters.[7, 8] Our objective was to determine the pattern of insulin requirements necessary to achieve

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and maintain normoglycemia among patients with gestational diabetes mellitus by the use of memory-based meters, to assure accurate characterization of insulin requirements throughout pregnancy.

Material and methods

Identification of gestational diabetes. At our medical center all pregnant women are screened for gestational diabetes mellitus between 24 and 28 weeks' gestation. For women with known selective risk factors (obesity, age, previous infants large for gestational age [LGA], or previous gestational diabetes mellitus) screening occurs at the initial prenatal visit. Screening consists of a 50 gm oral glucose load. If the 1-hour plasma glucose value is >135 mg/dl, the patient is scheduled for a full 3-hour oral glucose tolerance test, to be administered within between 3 and 7 days. All subjects are diagnosed in accordance with the criteria established by O'Sullivan and Mahan.[9] To confirm the diagnosis of gestational diabetes mellitus, all patients are given an oral glucose tolerance test (75 gm) between 2 and 6 months postpartum. Overt diabetes (type II) is defined according to the National Diabetes Data Group criteria.[10]

Study design. A prospective study of 57 patients with gestational diabetes mellitus who required insulin throughout the course of pregnancy was undertaken. To determine the initial assignment to insulin therapy, the patient had to satisfy one of two criteria. If either the fasting plasma glucose level during the oral glucose tolerance test or the 7-day overall mean blood glucose level determined by self-monitoring (immediately after the oral glucose tolerance test) was >94 mg/dl, insulin therapy was begun.[11]

Insulin algorithm. All subjects were classified according to body mass index (weight in kg/(height (m)^2) before pregnancy as obese (index >27.3) or nonobese. Obese patients received a diet of 25 kcal/kg current body weight, and nonobese patients were prescribed a diet of 35 kcal/kg body weight. The diet was calculated according to the ADA recommendations (carbohydrate 55%, protein 20%, fat 25%) and was synchronized with the insulin dose throughout the day. The insulin dose was calculated based on 0.7 U insulin per kilogram of body weight measured in pregnancy.[12]

Human insulin was administered to all patients and was divided into three injections: (1) regular and intermediate-acting insulin in the morning, (2) regular at dinner, and (3) intermediate acting at bedtime. The standard formula for the amount of insulin prescribed was two-thirds of all insulin in the morning (2:1, intermediate acting:regular) and one-third in the evening (1:1 regular:intermediate acting). If after 3 days of self-monitoring of blood glucose levels with insulin administration the overall glycemic profile had not reached the target zone (mean 75 to 95 mg/dl), insulin was to be increased at the rate of 15% to 20% for each dose. This procedure was to be repeated with a 10% to 15% increase in overall insulin dose. Thereafter, alteration in insulin dose was based on achievement of glucose target ranges for overall (75 to 95 mg/dl), fasting (60 to 80 mg/dl), preprandial (70 to 90 mg/dl), and postprandial (<120 mg/dl) levels.

Blood glucose evaluation. After diagnosis all patients were taught the self-monitoring of blood glucose levels through the use of a reflectance meter with an onboard memory. To assure maintenance of self-monitoring skills, each patient was retested at alternate clinic visits. The onboard memory in the meter can record up to 300 readings with corresponding time and date and was used to verify the blood glucose values obtained during the self-monitoring of blood glucose levels. Meter data were transmitted to a microcomputer for calculation of hourly, daily, and periodic means, as well as display of all glucose values, frequency distribution of glucose values, and measurement of standard deviation by periods within a day. Additionally, ambulatory glucose profiles were produced to assess typical daily glycemic patterns.[13] The self-monitoring blood glucose levels protocol requested 7 tests per day while fasting, before and after eating, and at bedtime. At each biweekly clinic visit the data from the reflectance meter were offloaded into a microcomputer for analysis of ambulatory glycemia.

Method of analysis. For the purpose of analysis all data were divided into three periods: Period 1, baseline (before treatment); period 2, from initiation of treatment (when insulin administration is based on "calculated" dose) to achievement of therapeutic goal; and period 3, maintenance of target glucose level. Pearson's correlation coefficient was calculated for the relationships between insulin requirements at each period. Student's t test was used to determine whether there was a significant difference in calculated and actual insulin doses.

Results

Patient characteristics. The 57 patients with gestational diabetes mellitus participating in the study had a mean age at entry of 28 ± 4 years. Fifty-seven percent of the subjects were obese according to the prepregnancy body mass index criteria. Mean gestational age at diagnosis was 26 ± 6 weeks. Distribution of gestational age showed that the earliest two patients were discovered between 18 and 20 weeks' gestation, with an additional 11 subjects identified by 23 weeks' gestation. Most women (30) were diagnosed between 24 and 28 weeks' gestation, with the remainder diagnosed by 33 weeks' gestation. The oral glucose tolerance test results were as follows: fasting, 109 ± 17 mg/dl;

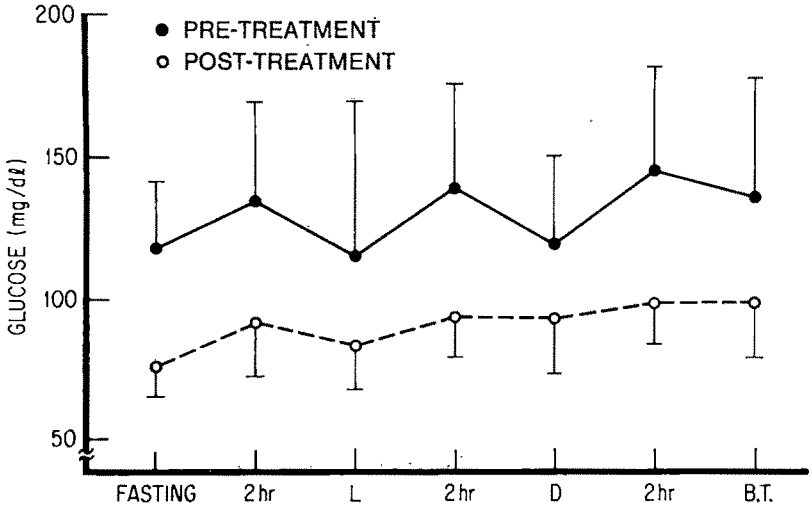


Fig. 1. Representation of glycemic control before and after treatment. This represents the metabolic control in periods I and II. *Significant difference ($p < 0.01$) before and after treatment at each time period.

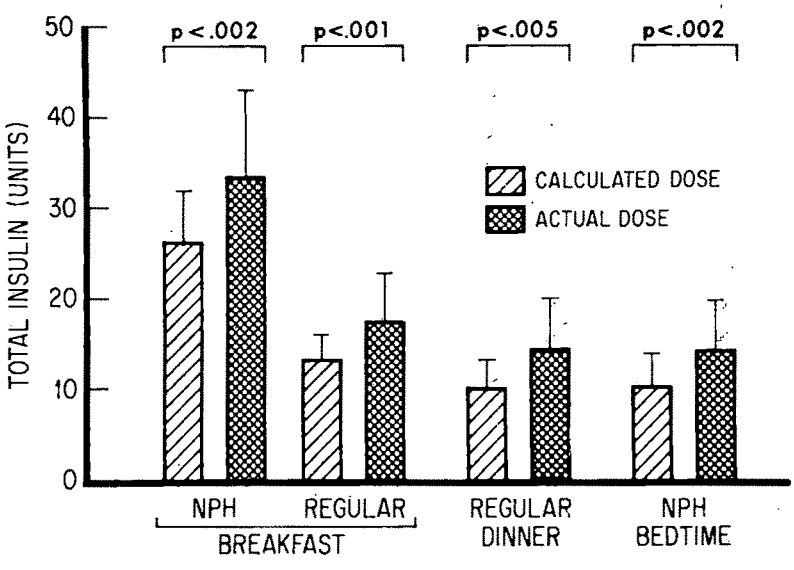


Fig. 2. Calculated versus actual insulin dose during period 2. *NPH* = Intermediate-acting insulin.

1 hour, 205 ± 33 mg/dl; 2 hours, 200 ± 43 mg/dl; and 3 hours, 148 ± 38 mg/dl. Average time of delivery was at 39 ± 2 weeks.

Postpartum evaluation by oral glucose tolerance test given between 2 and 6 months revealed no overt diabetes among the study group. The oral glucose tolerance test resulted in the following values: fasting, 99 ± 13 mg/dl; 1 hour, 156 ± 35 mg/dl; 2 hours, 122 ± 22 mg/dl, and 3 hours, 92 ± 23 mg/dl.

Period 1. Fig. 1 shows the overall level of glycemia for all study patients before the start of treatment (7 to 10 days). The mean blood glucose level was calculated as 139 ± 38 mg/dl. Variability in control was measured by the coefficient of variation (29%). In general, values after eating were higher than those before eating. Bedtime values showed the greatest deviation from the overall value. At the end of this period 52 subjects were assigned to insulin based on the oral glucose tolerance test fasting values and the mean blood glucose values for the period. Five additional patients were placed on insulin therapy due only to a mean blood glucose level >94 mg/dl for the baseline period.

Period 2. Fig. 2 shows the initially calculated insulin dose of 56 ± 10 U (based on the 0.7 U/kg formula) compared with the dose of 76 ± 14 U administered at the end of period 2 (achievement of glucose target). For the prebreakfast, predinner, and bedtime insulin doses, we found that a significantly higher amount of

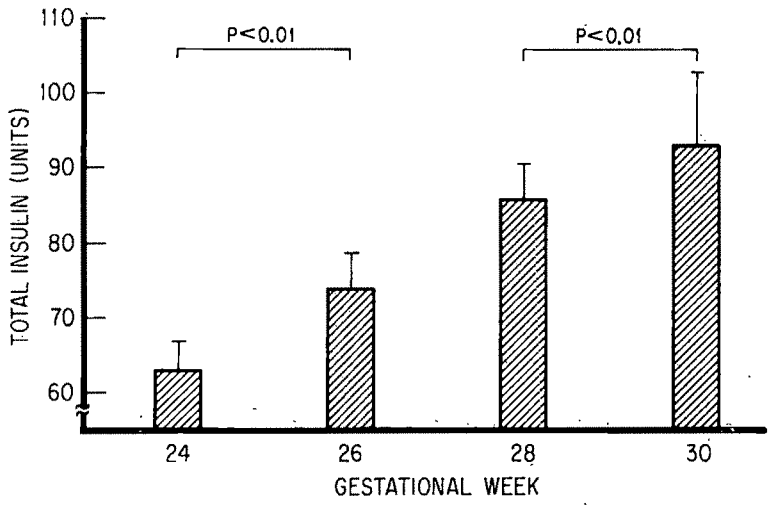


Fig. 3. Insulin requirements for 33 subjects in the early part of period 3.

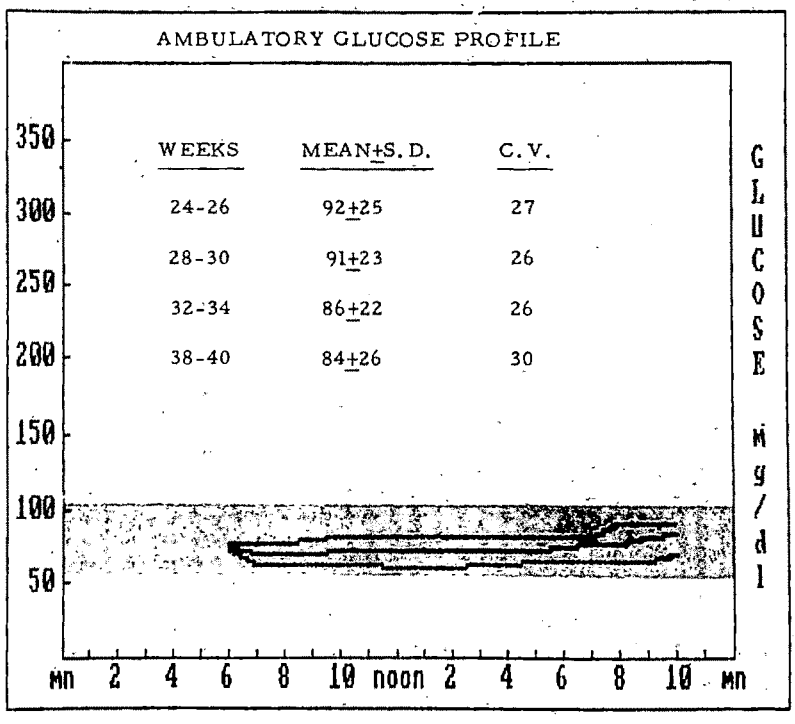


Fig. 4. Ambulatory glucose profile of patients throughout pregnancy. The computer printout from the memory-based reflectance meter summarizes the biweekly ambulatory glucose control based on self-monitoring data. The graph shows a typical pattern (from 6 AM to 10:30 PM) of gestational diabetes mellitus in treatment. The curves represent the median (middle) and variability (seventy-fifth and twenty-fifth percentiles) glucose values. The *shaded area* identifies the normal target range for glucose values throughout the day. *CV* = Coefficient of variation.

insulin was prescribed than calculated by the formula. During this period, glucose levels decreased significantly (from 139 ± 38 to 95 ± 21 mg/dl), with a comparable narrowing of variability (from 29% to 22%; Fig. 1). Summary statistics for this transitional period, which averaged 7 ± 2 days, revealed that the patients tested 6.5 ± 1 times a day and that overall insulin requirements increased by 36% ($p < 0.01$).

Period 3. Examination of the change in insulin requirements that occurred among the 33 patients who

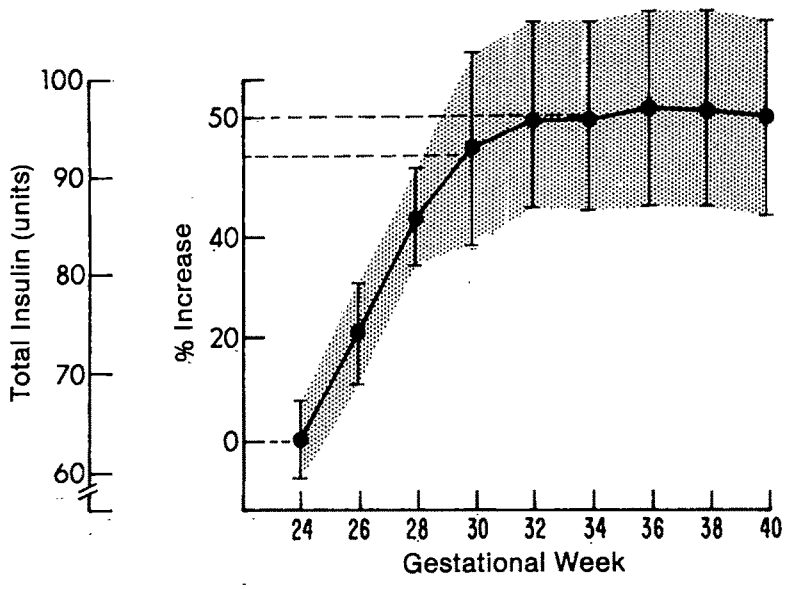


Fig. 5. Insulin requirement throughout pregnancy for 57 subjects. Total insulin required throughout pregnancy and percent increase in insulin are measured along the y axis. The period of 24 to 30 weeks' gestation shows the greatest change in absolute dose and percent, while the period from 32 to 40 weeks' gestation shows the greatest stability. The *shaded area* represents 1 SD.

Table I. Insulin requirements and correlation coefficients

	24 ± 2 versus 32 ± 3 wk			*32 ± 3 versus 39 ± 2 wk*		
	r	R^2	*p*	*r*	R^2	*p*
Morning						
Intermediate-acting	0.60	0.36	<0.001	0.98	0.97	<0.0001
Regular	0.55	0.30	<0.005	0.99	0.98	<0.0001
Evening						
Regular	0.63	0.40	<0.001	0.80	0.64	<0.0001
Intermediate-acting (10 PM)	0.47	0.22	<0.02	0.99	0.98	<0.0001

were at 24 ± 2 weeks, gestational age at entry into period 3 revealed a constant increase in insulin dose to maintain the target blood glucose levels. This increase was significant between 24 to 26, 26 to 28, and 28 to 30 weeks' gestation (Fig. 3). Overall insulin requirements increased by 47% during the early part of period 3. Regression analysis revealed a correlation ($r = 0.57$; $p < 0.001$) between the predictability of the 32 weeks' gestation insulin dose from the 24 week insulin dose. This association was shown to occur for each type of insulin. The fasting, preprandial and postprandial, and bedtime glucose levels did not significantly change during this period. Overall metabolic control was calculated as 92 ± 25 mg/dl in the early part of period 3.

Examination of the change in insulin requirements during the latter half of period 3, when all 57 subjects achieved the target glucose range, revealed an average blood glucose level of 91 ± 23 mg/dl, which did not significantly change throughout this period. Analysis by ambulatory glucose profile of daily glycemic control for all patients showed that blood glucose values remained within a narrow target range, with no significant excursions throughout the day (Fig. 4). Furthermore, we found no significant increase in insulin requirements during this time (Fig. 5). There was a strong positive correlation ($r = 0.99$; $p < 0.0001$) between the overall insulin dose at 32 and 40 weeks' gestation. Additionally, a correlation of $r = 0.99$ ($p < 0.0001$) was calculated for the predictability of morning insulin requirements and $r = 0.80$ to 0.99 ($p < 0.0001$) for evening insulin requirements when the thirty-second and thirty-ninth weeks were examined. $r = 0.62$ ($p < 0.001$) for regular insulin at dinner (Table I).

We then examined the different insulin requirements for the obese (body mass index >27.3) and nonobese study subjects. For the obese subjects and actual insulin requirements were 0.8 U/kg and for the nonobese sub-

jects it was 0.9 U/kg, with no significant difference between them. However, there was a significant difference in variability, as measured by the coefficient of variation (45% versus 25%; $p < 0.01$).

Comment

Gestational diabetes mellitus is a disease whose characteristic is carbohydrate intolerance with first onset or recognition during pregnancy.[14] It encompasses a heterogeneous array of insulin secretion and action disorders that manifest themselves in varying levels of metabolic control. The clinical importance of gestational diabetes mellitus lies in its relationship to adverse perinatal and neonatal outcome (mainly macrosomia and stillbirth) when these individuals are not treated or are maintained in poor metabolic control; in its masking of pregestational diabetes; and in an increased risk of developing type II diabetes.

Two features of the current study make it unique. First, it included women with gestational diabetes mellitus characterized by varying degrees of carbohydrate intolerance and shown postpartum not to have overt (type II) diabetes. With these subjects we were able to evaluate the insulin requirements needed to achieve near-normal levels of glycemic control in gestational diabetes mellitus. Second, utilization of memory-based reflectance meters (providing verified blood glucose data) permitted us to characterize ambulatory glycemic control throughout the pregnancy. This, in turn, enabled us to aggregate glucose data efficiently and evaluate the level of glycemic control and its association to insulin requirements throughout the pregnancy of patients with gestational diabetes mellitus.

Our study revealed that normoglycemia in women with gestational diabetes could be achieved and maintained for prolonged periods in ambulatory prenatal care. The achievement and maintenance of near-normal glycemia was possible by accurately predicting the pattern of insulin requirement and individual insulin adjustments throughout the course of gestational diabetes.

Although the role of insulin requirement in pregestational diabetes (types I and II) was previously studied,[12, 15] its role in gestational diabetes is not well established. In individuals with type II diabetes, there is an almost linear rise in insulin dose between the second and the ninth months, with a twofold increase in daily insulin requirement.[16] In contrast, we found that patients with gestational diabetes mellitus have a gradual and significant increase in insulin requirements until the thirtieth week of gestation and thereafter stabilization occurs. Furthermore, during the latter half of the third trimester (30 to 39 weeks) the variability in insulin dose remains unchanged. Additionally, we found that the total insulin dose for subjects with gestational diabetes mellitus (91 ± 10 U) was greater than the total dose previously reported for individuals with type I diabetes (70 ± 18 U).

As with any type of therapy, the potential benefits of treatment must be weighed against the potential risks. For instance, the selection of a threshold for insulin administration (>94 mg/dl) was predicated on our earlier study, which showed a high incidence of macrosomia when diet-only regimens were prescribed to patients with fasting plasma glucose levels between 95 and 105 mg/dl.[11] Furthermore, we noted that the standard "calculated" insulin dose at the start of treatment had to be significantly increased to reach the target glycemic range. Despite the potential for low blood glucose levels when insulin administration is used to tighten metabolic control, no episodes of hypoglycemia occurred in any of the patients during this period.

The calculated dose (0.7 U/kg), based on previous studies[12, 16] of the insulin requirements in pregestational diabetes (type I), provided an appropriate starting point for insulin administration, because it improved control within two adjustments without the risk of hypoglycemia. Consequently, we were able to identify an algorithm that provided an appropriate and safe method of rapidly achieving the target glucose range.

We found that the relative instability in glucose metabolism could only be corrected with increasing levels of insulin during period 2 and the early stages of period 3. Once glucose metabolism stabilized, insulin dosage remained relatively constant. Thus we conclude that frequent clinic visits and adjustments in insulin doses are mandatory for accurate clinical decision-making during the early stages of gestational diabetes mellitus. This in turn will, we suspect, decrease adverse perinatal and neonatal outcomes. Additionally, we noted that in the late stage of period 3, virtually no variation in insulin requirements exists. This suggests that a less pronounced effort is required for maintaining the adherent patient in good metabolic control during this period.

Our data suggest that the mechanism by which insulin requirements change may be related to human placental lactogen secretion, because the insulin dose and human placental lactogen curves coincide. They both tend to rise in the second trimester and plateau at 32 to 35 weeks' gestation. Because human placental lactogen has been identified as a major diabetogenic factor in pregnancy, it is reasonable to assume that the glucose profile would be affected in gestational diabetes mellitus. Therefore, the identification of gestational diabetes mellitus may occur as early as 18 weeks' gestation and as late as 35 weeks.

In summary, the main changes in insulin requirements are evident before 30 weeks' gestation; these requirements do not increase thereafter; and the em-

phasis on ambulatory self-monitoring of blood glucose levels and readjustment of the insulin dose should be maximized within this interval.

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Status of infants at birth and risk for adverse neonatal events and long-term sequelae: A study in low birth weight infants

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We evaluated the relationship of infant status at birth to neonatal morbidity and long-term development in 246 low birth weight infants (600 to 1750 gm). Nineteen percent of infants had 1-minute Apgar scores of ≤3, and 8% had an umbilical artery pH of ≤7.2. Acidosis was associated with an increased risk of grade 3 to 4 intracranial hemorrhage (odds ratio = 3.3). Low 1-minute Apgar score was associated with an increased risk of death (odds ratio = 4.8). Grade 3 to 4 intracranial hemorrhage was a strong risk factor for cerebral palsy among survivors (odds ratio = 16.1), as was low 1-minute Apgar score (odds ratio = 2.9). Only 15% of cases of cerebral palsy in this study were associated with acidosis at birth. (Am J Obstet Gynecol 1987;157:676-9.)

Key words: Apgar score, cord artery pH, intracranial hemorrhage, cerebral palsy

The relationship of intrapartum events to infant status at birth and subsequent neonatal and infant morbidity remains controversial. Although the available data have been reviewed recently,[1] this publication dealt primarily with term infants. Since infants of low birth weight are at high risk for adverse outcomes, the present study was undertaken to evaluate the relationship of infant status at birth to the risk for neonatal mortality and morbidity, including abnormal development.

Material and methods

The study was conducted at the University of Washington, Seattle, and Grace Hospital, Vancouver, British Columbia, Canada, from November 1981 to March 1, 1985.

The study group consisted of 246 low birth weight infants weighing from 600 to 1750 gm. These infants were all singleton, in cephalic presentation, and products of premature labor at 26 to 32 weeks' gestation. Multiple pregnancy, noncephalic presentation, antenatal hemorrhage, recognizable malformations, and infants delivered before the onset of labor were excluded from the study. All patients were part of a prospective study of electronic fetal heart rate monitoring that included care by a study nurse on a one-to-one basis. Labors were managed by house officers with attending physician supervision. Protocols used for fetal heart rate monitoring and labor are described in detail elsewhere.[2]

One-minute Apgar scores and umbilical cord arterial pH were used as indicators of the infant status at birth. At the time of delivery, 1- and 5-minute Apgar scores were assigned by the senior pediatrician in attendance. A 1-minute Apgar score of ≤3 was considered to be significant neonatal depression. A doubly clamped segment of umbilical cord was obtained for complete blood gas analysis. An umbilical cord arterial pH of ≤7.20 was considered to indicate acidosis. This value was chosen because a scalp pH of ≤7.20 would have prompted immediate delivery by the labor management protocol in use during this study.[2] At 76 to 92 hours of age, all infants underwent a cranial ultrasound examination to detect intracranial hemorrhage. Intracranial hemorrhage was graded 1 to 4 according to severity.[3] At 4, 8, and 18 months' corrected age, survivors of the neonatal period had developmental testing, by means of the Bayley scale of infant development.[4] At 18 months' corrected age, developmental pediatricians classified infants as either definitely having cerebral palsy or not having cerebral palsy.

Correlation coefficients were calculated to assess the association between Apgar score, cord arterial pH, and birthweight. Odds ratios and 95% confidence limits were used to estimate risk of adverse infant status at birth for late neonatal and infant morbidity. The χ^2 analysis was used to test for statistical significance. Since birth weight was a confounding variable, a logistic regression was used to control for birth weight in the

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Table I. Umbilical cord arterial pH versus 1-minute Apgar score among 199 infants weighing 600 to 1750 gm

Umbilical artery pH	*Apgar score at 1 min*				*Total No.*
	≤3		*>3*		
	n	*%*	*n*	*%*	
≤7.2	4	2	12	6	16
>7.2	34	17	149	75	183
Total	38		161		199

r = 0.23, p = 0.001.

Table II. One-minute Apgar score and morbidity/mortality by 200 gm birth weight increments among 246 infants weighing 600 to 1750 gm

Birth weight (gm)	*n*	*Intracranial hemorrhage, grade 3 to 4*	*Deaths*	*Cerebral palsy*
Apgar score >3				
501-700	1	—	—	—
701-900	10	1	2	—
901-1100	23	7	4	2
1101-1300	42	5	1	3
1301-1500	44	4	2	6
1501-1750	53	—	—	3
Subtotal	173	17 (10%)	9 (5%)	14 (10%)*
Apgar score ≤3				
501-700	8	3	5	1
701-900	22	7	11	1
901-1100	15	8	5	3
1101-1300	12	—	1	—
1301-1500	10	1	4	1
1501-1750	6	—	—	3
Subtotal	73	19 (26%)	26 (36%)	9 (20%)†
Total	246	36 (15%)	35 (14%)	23 (12%)‡

*Denominator = 142 infants evaluated.
†Demoninator = 45 infants evaluated.
‡Denominator = 189 infants evaluated.

analysis. However, odds ratios, both crude and adjusted for birth weight, are given for comparison. Simultaneous control of Apgar score and umbilical artery pH did not change the risk estimate significantly for any of the outcome measures (intracranial hemorrhage, death, cerebral palsy) analyzed.

Results

There were 35 deaths (14%) among the 246 infants studied. One hundred ninety-nine infants (80%) had cord blood gas analysis performed at the time of delivery. There were 212 surviving infants. Of those, 187 (88%) had at least one follow-up examination.

There was a statistically significant but low association between birth weight and 1-minute Apgar score ($r = 0.4$, $p < 0.0001$). There was a lower but also statistically significant association between birth weight and umbilical cord arterial pH ($r = 0.23$, $p < 0.0001$).

Among the 199 infants who had umbilical cord blood gas analysis performed, the association between umbilical cord arterial pH and 1-minute Apgar score was statistically significant but not strong ($r = 0.23$, $p = 0.001$). Overall, 19% of infants had a 1-minute Apgar score of ≤3 and 8% of infants had an umbilical cord arterial pH of ≤7.20. Seventy-five percent of the 199 infants had 1-minute Apgar scores >3 and umbilical artery pH >7.20. Two percent of infants had a depressed Apgar score at 1 minute and a low cord artery pH. Only 10% of low Apgar scores were associated with an umbilical cord arterial pH of ≤7.20, whereas 75% of acidotic infants (12 of 16) were not depressed at birth (Table I).

Overall, 36 of 246 infants (15%) developed grade 3 to 4 intracranial hemorrhage (Table II). Twenty-six percent of infants (19 of 73) with 1-minute Apgar scores of ≤3 developed grade 3 to 4 intracranial hemorrhage

Table III. Umbilical artery pH and morbidity/mortality by 200 gm birth weight increments among 199 infants weighing 600 to 1750 gm

Birth weight (gm)	*n*	*Intracranial hemorrhage, grade 3 to 4*	*Deaths*	*Cerebral palsy*
Umbilical artery pH > 7.20				
501-700	6	2	3	1
701-900	17	4	7	—
901-1100	18	5	3	2
1101-1300	46	4	2	2
1301-1500	39	1	4	6
1501-1750	53	—	—	5
Subtotal	179	16 (9%)	19 (11%)	16 (11%)*
Umbilical artery pH ≤ 7.20				
501-700	1	1	1	—
701-900	5	1	3	—
901-1100	7	3	2	1
1101-1300	2	—	—	1
1301-1500	5	2	—	1
1501-1750	—	—	—	—
Subtotal	20	7 (35%)	6 (30%)	3 (14%)†
Total	199	23 (12%)	25 (13%)	19 (12%)‡

*Denominator = 142 infants evaluated.
†Denominator = 14 infants evaluated.
†Denominator = 156 infants evaluated.

Table IV. Number of infants with grade 3 to 4 intracranial hemorrhage and cerebral palsy among 187 infants weighing 600 to 1750 gm

Intracranial hemorrhage, grade 3 to 4	*Cerebral palsy*			
	No (n)	*Yes*		*Total*
		n	*%*	
No	149	13	8	162
Yes	15	10	40	25
Total	164	23	12	187

compared with 10% (17 of 174) of infants with 1-minute Apgar scores >3 (crude odds ratio = 3.3, 95% confidence interval = 1.5 to 7.1; adjusted odds ratio = 1.5, 95% confidence interval = 0.6 to 3.3). The odds ratio is not statistically significant when adjusted for birth weight. Of the infants who had cord blood gas analysis, 23 of 199 (12%) developed grade 3 to 4 intracranial hemorrhage (Table III). Thirty-five percent (seven of 20) of infants with an umbilical cord arterial pH of ≤7.20 had grade 3 to 4 intracranial hemorrhage, compared with 9% (16 of 179) of those with a cord arterial pH >7.20 (crude odds ratio = 5.5, 95% confidence interval = 1.6 to 17.3; adjusted odds ratio = 3.3, 95% confidence interval = 1.1 to 10.3). The odds ratio is statistically significant when adjusted for birth weight.

A strong correlation was found between the risk of death and 1-minute Apgar score of ≤3. Thirty-six percent of infants (26 of 73) with low Apgar scores at 1 minute died, compared with 5% (nine of 174) of those infants with 1-minute scores >3 (crude odds ratio = 10.1, 95% confidence interval = 4.2 to 25.2; adjusted odds ratio = 4.8, 95% confidence interval = 1.8 to 11.9). Twenty-five of 199 infants (13%) who had cord blood gas analysis died. Thirty percent (six of 20) of infants with an umbilical artery pH of ≤7.2 died, compared with 11% of those with an umbilical artery pH >7.2 (crude odds ratio = 3.6; 95% confidence interval = 1.0 to 11.5; adjusted odds ratio = 1.8; 95% confidence interval = 0.6 to 6.1).

After the 18-month infant examination, there were 23 cases of cerebral palsy among the 187 infants (12%) who had developmental testing. Twenty percent of infants (nine of 45) with a 1-minute Apgar score of ≤3 developed cerebral palsy, compared with 10% (14 of 142) of those with 1-minute Apgar scores >3 (crude odds ratio = 2.6, 95% confidence interval = 1.0 to 6.9; adjusted odds ratio = 2.9, 95% confidence interval = 1.1 to 7.9). Twenty-one percent of infants (three of 14) with an umbilical artery pH of ≤7.20 developed cerebral palsy, compared with 11% of infants (16 of 142) with an umbilical artery pH >7.20 (crude odds ratio = 2.3, 95% confidence interval = 0.4 to 10.3; adjusted odds ratio = 2.6, 95% confidence interval = 0.6 to 11.0).

The risk of cerebral palsy was most strongly associated with grade 3 to 4 intracranial hemorrhage (Table IV). Thirteen percent of surviving infants who had developmental testing (25 of 187) had a grade 3 to 4

intracranial hemorrhage. Forty percent of surviving infants (10 of 25) with grade 3 to 4 intracranial hemorrhage developed cerebral palsy, compared with 8% (13 of 162) of those without grade 3 to 4 intracranial hemorrhage (crude odds ratio = 8.3, 95% confidence interval = 3.2 to 22.0; adjusted odds ratio = 16.1, 95% confidence interval = 4.6 to 57.1).

Comment

Our study was composed of premature infants (26 to 32 weeks) of low birth weight (600 to 1750 gm). Premature infants with birth weights in this range are known to be at high risk for neonatal morbidity or death as well as abnormal development, when compared with term infants of normal birth weight. Few studies, especially of low birth weight infants, have longitudinally evaluated infants from birth with cord blood gas data, cranial ultrasound examinations, and developmental testing. Since study design excluded many pregnancies with antenatal risk factors, our study population consisted primarily of patients with idiopathic premature labor, including premature rupture of the membranes. Thus the infant's status at birth in our study may have been less affected by antenatal factors than would have been true in a cross-sectional study of all premature infants.

While one in five infants had a 1-minute Apgar score of ≤3, fewer than one in 10 infants had an umbilical cord artery pH of ≤7.20. The correlation between 1-minute Apgar score and umbilical artery pH, while statistically significant in our study, was not a strong association. Others have noted the low association between Apgar scores and umbilical artery pH in term infants[5] as well as preterm infants.[6] Only 10% (four of 38) of low Apgar scores in our study were associated with acidosis. The term perinatal asphyxia is frequently applied to infants with low Apgar scores, especially when umbilical gas data are not available. The results of our study, as well as others,[5,6] suggest that this practice is inaccurate, at the least, for both term and premature infants. We suggest the term perinatal asphyxia should be used only with biochemical evidence of same.

A low 1-minute Apgar score was associated with almost a fivefold increased risk for death when adjusted for birth weight. In 75% of all deaths in our study, the 1-minute Apgar score was ≤3. Neonatal depression was a strong risk factor for death, but this was not related to acidosis. This risk remained after adjustment for birth weight; thus the lowering of Apgar scores that resulted from very small infants cannot be the explanation. Generalized central nervous system depression at birth could explain the lack of correlation with acidosis as well as the increased risk for death in these infants; however, this hypothesis cannot be evaluated with the available data.

Acidosis (cord artery pH < 7.20) was associated with a threefold increased risk for grade 3 to 4 intracranial hemorrhage, an association unlikely to be due to chance or differences in birth weight. However, this accounted for only 30% of all cases of grade 3 to 4 intracranial hemorrhage in our study. We noted a trend toward increasing risk of intracranial hemorrhage with worsening acidosis. The risk of grade 3 to 4 intracranial hemorrhage was 50% in our study if the cord artery pH was ≤7.15; if the pH was 7.15 to 7.20, the rate was the same as in infants with an umbilical artery pH >7.20.

Grade 3 to 4 intracranial hemorrhage was the strongest risk factor for cerebral palsy. More than 40% of infants with cerebral palsy in our study had a major intracranial hemorrhage. Infants with grade 3 to 4 intracranial hemorrhage had a sixteenfold increased risk for cerebral palsy, when adjusted for birth weight, compared with infants with grade 1 to 2 intracranial hemorrhage or without intracranial hemorrhage. Low 1-minute Apgar score was also associated with a statistically significant risk (almost threefold) for cerebral palsy. While a statistically significant correlation between acidosis and cerebral palsy could not be demonstrated, given the strong association between acidosis and intracranial hemorrhage and between intracranial hemorrhage and cerebral palsy, our study may not have been large enough to detect a true difference. However, most cases of cerebral palsy in our study of premature infants were unrelated to acidosis at birth. Only 15% of the cases of cerebral palsy in our study were associated with an umbilical artery ≤pH of 7.20. This suggests that factors other than birth asphyxia may have important roles in the causes of cerebral palsy among premature infants, and our data are in agreement with the concept that cerebral palsy has multifactorial causes in premature infants.

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A classification of hypertension in pregnancy based on Doppler velocimetry

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We studied 136 pregnant women with hypertension with Doppler velocimetry of the uterine and umbilical arteries. The patients were classified into four groups according to the values of the systolic/diastolic ratios. The first group had normal ratios in both the umbilical and uterine arteries. The second group had elevated umbilical ratios and normal ratios in the uterine arteries. The third group had elevated uterine artery systolic/diastolic ratios with normal umbilical artery values, and the fourth group had elevated systolic/diastolic ratios in both vessels. Women in the group with normal ratios in both the umbilical and uterine arteries were delivered of infants with a birth weight (means ± SD) of 3261 ± 522 gm and gestational age of 39 ± 2 weeks. The values for the three groups with abnormal velocimetry were: those with elevated umbilical ratios and normal ratios in the uterine arteries: body weight = 2098 ± 811 gm, gestational age = 35.7 ± 3.2 weeks; those with elevated uterine artery ratios with normal umbilical artery values: 2464 ± 722 gm, gestational age = 36.3 ± 3 weeks; and those with elevated systolic/diastolic ratios in both vessels: body weight = 1627 ± 697 gm, gestational age = 33.3 ± 2.7 weeks ($p < 0.01$; $p < 0.001$). There were 27 small for gestational age infants delivered during this study. Doppler velocimetry studies were abnormal in 26 of them (96%). Results show that Doppler-derived vascular patterns correlate well with normal and adverse perinatal outcome. A description of the uterine and umbilical systolic/diastolic ratios should be part of the clinical evaluation of all pregnant women with hypertension. This should lead to better treatment protocols and improved clinical outcome. (Am J Obstet Gynecol 1987;157:680-5.)

Key words: hypertension, pregnancy, Doppler

Hypertensive disorders in pregnancy are difficult to distinguish. In many diseases hypertension can be a manifestation in women in the reproductive age group. Their effect on pregnancy is variable. The rates of preterm birth, small for gestational age infants, and perinatal death are increased in pregnancies complicated by hypertension.[1, 2] According to current recommendations, all pregnant women with hypertension undergo serial ultrasonographic examinations and weekly fetal heart rate testing or biophysical profiles to identify the fetus at risk for hypoxia and acidosis.[3] Elective preterm delivery is frequently advocated for hypertensive women in an attempt to improve neonatal outcome, although this frequently incurs the risks of cesarean section and prematurity.

Hypoxic complications in fetuses of hypertensive women are thought to be the result of vascular lesions in the utero-placental circulation with resultant decrease in blood flow.[4] Doppler velocimetry is a technique that is useful for assessing blood flow and vascular resistance and thereby offers the promise of better defining the fetal and maternal risk in pregnancy hypertensive syndromes.[5-7]

The purpose of this study was to examine Doppler velocimetry of the umbilical and uterine arteries in women whose pregnancies were complicated by hypertension. Women were classified by vascular patterns. Results show that Doppler-derived vascular patterns correlate well with normal and adverse perinatal outcome.

Material and methods

One hundred thirty-six women seen in the antepartum testing units of Winthrop-University Hospital, Stony Brook University Hospital, Bronx Municipal Hospital Center, and Westchester County Medical Center because of hypertension make up the sample studied. Doppler waveforms were obtained from the uterine and umbilical arteries. The equipment used was the Angioscan (Ultrasonix, Mt. Vernon, New York) or the Multigon 500 A (Multigon Industries, Mt. Vernon,

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Table I. Maternal parameters versus Doppler-derived vascular patterns

	Nn (n = 66)	*Na (n = 27)*	*An (n = 12)*	*Aa (n = 31)*
Age (yr)	29 ± 7	27 ± 6	24 ± 7	27 ± 6
% Nulliparous	57	52	80	63
MAP (second trimester)	97 ± 12	95 ± 13	105 ± 21	96 ± 21
% Abnormal platelets	0	26*	13	0
Uric acid (mg/dl)	5.6 ± 1.1	6.5 ± 2.2	6.0 ± 1.4	6.5 ± 1.8*
% Proteinuria	24	71†	75†	86‡
Uterine artery S/D	2 ± 0.3	2.1 ± 0.3	3.4 ± 0.9‡	4.1 ± 1.5‡

Nn, group with normal uterine and umbilical artery ratios; Na, group with normal uterine and abnormal umbilical artery ratios; An, group with normal umbilical and abnormal uterine artery ratios; Aa, group in whom both ratios were abnormal; MAP, mean arterial pressure; S/D, systolic/diastolic.

*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

Table II. Fetal and neonatal parameters versus Doppler-derived patterns

	Nn (n = 66)	*Na (n = 27)*	*An (n = 12)*	*Aa (n = 31)*
Birth weight (gm)	3261 ± 522	2098 ± 811	2464 ± 722	1627 ± 697
Gestational age (wk)	39 ± 2	35.7 ± 3.2	36.3 ± 3	33.3 ± 2.7
Delivery <37 wk (%)	11	61	67	84
% SGA	2	29	17	51
% C/S for fetal distress	8	39	8	62
% NICU	12	68	50	89
Umbilical artery S/D	2.4 ± 0.3	4.2 ± 1.1	2.5 ± 0.3	4.6 ± 1.1

Nn, group with normal uterine and umbilical artery ratios; Na, group with normal uterine and abnormal umbilical artery ratios; An, group with normal umbilical and abnormal uterine artery ratios; Aa, group in whom both ratios were abnormal; SGA, small for gestational age; C/S, cesarean section; NICU, neonatal intensive care unit; S/D, systolic/diastolic.

New York) spectral analyzer. These are continuous-wave Doppler systems. The maximum Doppler shift during systole is divided by the maximum shift during end diastole to give the systolic/diastolic ratio.[8, 9]

Pattern recognition was used to identify the waveforms as being uterine or umbilical. The umbilical artery was studied from at least three different angles and the systolic/diastolic ratios were averaged. Two readings of each uterine artery were recorded and averaged at each study.

Women were studied serially from time of referral until time of delivery. The majority of women underwent two or more studies. The reported values are the mean umbilical artery systolic/diastolic ratio after 30 weeks and the mean uterine artery systolic/diastolic ratio after 26 weeks. An average umbilical systolic/diastolic ratio of 3.0 or more after 30 weeks was used as a cutoff. We have used this value as the most sensitive and specific for the detection of intrauterine growth retardation.[9] The mean uterine systolic/diastolic ratio from 26 weeks to term is 2.0 with an SD of 0.3, and once again the data in this series corroborate this figure (Table I). The mean umbilical systolic/diastolic ratio after 30 weeks in a normal population is 2.4 with an SD of 0.3, which is the same as that of the group with normal ratios in both the umbilical and uterine arteries in this series (Table II). A uterine systolic/diastolic ratio >2.6 after 26 weeks had previously been shown to be associated with adverse perinatal outcome[10] (Figs. 1 and 2).

Patients were then divided into four groups based on velocimetry: (1) those with normal uterine and umbilical artery systolic/diastolic ratios, (2) those with normal uterine artery systolic/diastolic ratios and abnormal umbilical artery systolic/diastolic ratios, (3) those with normal umbilical artery systolic/diastolic ratios and abnormal uterine artery systolic/diastolic ratios, and (4) those in whom both systolic/diastolic ratios were abnormal.

The patients were also classified by clinical diagnosis. Chronic hypertension was defined as a mean arterial pressure of >90 mm Hg at 20 weeks. Pregnancy-induced hypertension was defined as a rise in systolic blood pressure >30 mm Hg or the diastolic blood pressure >15 mm Hg over the first trimester blood pressure determinations that persisted after 6 hours of bed rest. Preeclampsia was diagnosed when pregnancy-induced hypertension was accompanied by proteinuria (>300 mg in a 24-hour urine collection or >3+ on dipstick). If the patient's prenatal records were not available, the attending physician's discharge diagnosis was taken as the clinical diagnosis.

An abnormal nonstress test result was defined as a fetal heart rate score of 7 or less.[11] Small for gestational age diagnosis was defined as ≤the tenth percentile, Denver classification.

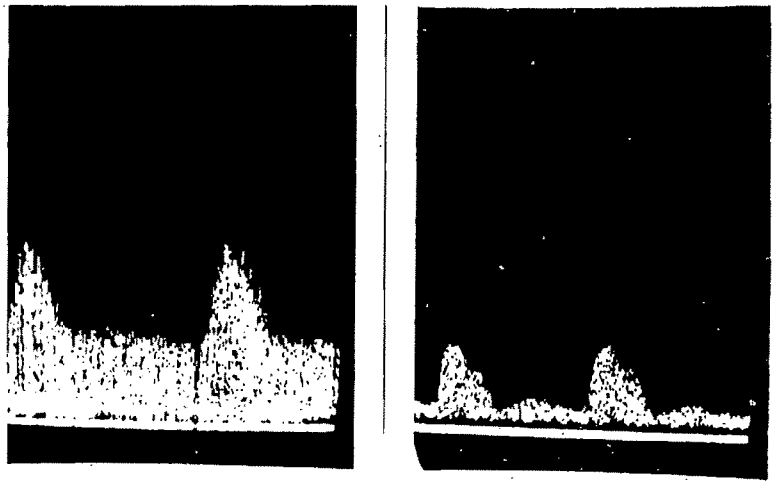


Fig. 1. Doppler images of uterine artery waveforms. (*S/D*, systolic/diastolic.)

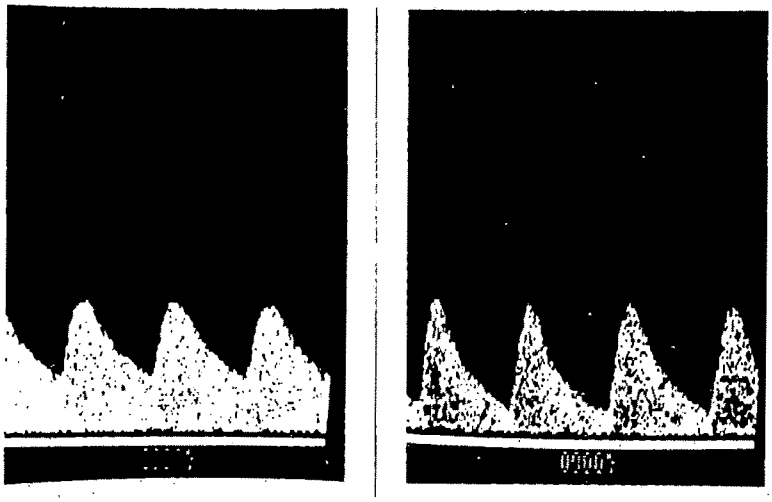


Fig. 2. Doppler images of umbilical artery waveforms. (*S/D*, systolic/diastolic.)

All patients were managed by attending physicians or house staff with faculty supervision. The results of the Doppler studies were not used to influence clinical management.

Results

The various maternal parameters studied are listed in Table I. There was no difference in age, percentage of nulliparous women, or mean arterial pressure in the second trimester between the four groups. Thrombocytopenia was present in 26% of the group with normal uterine and abnormal umbilical artery ratios ($p < 0.05$) and 13% of the group with normal umbilical and abnormal uterine artery ratios (p not significant). It was not seen in the groups with normal uterine and umbilical artery ratios and those in whom both ratios were abnormal.

The mean uric acid level for the group with normal

Table III. Doppler-derived vascular patterns versus clinical diagnosis

	n	*Nn (n = 58)* (%)	*Na (n = 27)* (%)	*An (n = 12)* (%)	*Aa (n = 31)* (%)
Chronic hypertension	43	65	23	5	7
PIH	34	59	15	6	21
Preeclampsia	51	20	24	16	41

Nn, group with normal uterine and umbilical artery ratios; Na, group with normal uterine and abnormal umbilical artery ratios; An, group with normal umbilical and abnormal uterine artery ratios; Aa, group in whom both ratios were abnormal; PIH, pregnancy-induced hypertension.

uterine and umbilical artery ratios was 5.6. For the groups with normal uterine and abnormal umbilical artery ratios, those with normal umbilical and abnormal uterine artery ratios, and those in whom both ratios were abnormal the values were 6.5, 6.0, and 6.5, respectively. Although there appears to be a trend toward higher levels, there was a great deal of overlap among the individual values between all four groups. The only group in whom statistical significance was reached was the group in whom both ratios were abnormal.

Proteinuria was present in 24% of women in the group with normal uterine and umbilical artery ratios, 71% of those in the group with normal uterine and abnormal umbilical artery ratios, 75% of those in the group with normal umbilical and abnormal uterine artery ratios, and 86% of those in the group in whom both ratios were abnormal. A total of 77 women had proteinuria in this study sample. Sixty-one of them (77%) had abnormal Doppler velocity waveforms of the uterine or umbilical arteries.

The clinical condition of mother or fetus led to intervention in many of these pregnancies. In the normal-normal group, this occurred in one third of the women. It occurred 58% of the time in patients with normal uterine and abnormal umbilical artery ratios and those with normal umbilical and abnormal uterine artery ratios ($p < 0.05$) and 74% of the time when both ratios were abnormal ($p < 0.001$). In the latter group the predominant indication was an abnormal antepartum fetal heart rate pattern.

There were two cases of eclampsia seen during this study. Both of those women were in the group with normal uterine and abnormal umbilical artery ratios.

Table II summarizes fetal parameters studied. The birth weights and gestational ages of the four groups were as follows (means ± SD): group with normal uterine and umbilical artery ratios, 3261 ± 522 gm and 39 ± 2 weeks; group with normal uterine and abnormal umbilical artery ratios, 2098 ± 811 gm and 35.7 ± 3.2 weeks; group with normal umbilical and abnormal uterine artery ratios, 2464 ± 722 gm and 36.3 ± 3 weeks; and those in whom both ratios were abnormal, 1627 ± 697 gm and 33.2 ± 2.7 weeks. The incidence of preterm delivery in the four groups was as follows: group with normal uterine and umbilical artery ratios, 11%; group with normal uterine and abnormal umbilical artery ratios, 61%; group with normal umbilical and abnormal uterine artery ratios, 67%; and those in whom both ratios were abnormal, 84%.

Fetuses of women with abnormal uterine or umbilical systolic/diastolic ratios weighed less and were delivered earlier than those whose mothers had normal values. One neonate was small for gestational age in the group with normal uterine and umbilical artery ratios (2%). This infant was delivered normally at 40 weeks and weighed 2000 gm. The infant had no problems in the neonatal period. Twenty-nine percent ($p < 0.01$) of fetuses in the group with normal uterine and abnormal umbilical artery ratios were small for gestational age. Seventeen percent (p not significant) of fetuses in the group with normal umbilical and abnormal uterine artery ratios were small for gestational age and 51% ($p < 0.001$) of fetuses in those in whom both ratios were abnormal were small for gestational age.

Overall 27 small for gestational age infants were delivered during this study. Doppler velocimetry of either the umbilical or uterine arteries was abnormal in 26 of them (96%).

Fetal distress in labor occurred in 8% of the group with normal uterine and umbilical artery ratios, 39% ($p < 0.01$) of the group with normal uterine and abnormal umbilical artery ratios, 8% (p not significant) of the group with normal umbilical and abnormal uterine artery ratios, and 62% ($p < 0.001$) of those in whom both ratios were abnormal.

Twelve percent of neonates delivered of women in the group with normal uterine and umbilical artery ratios required admission to the neonatal intensive care unit and the percentages of the group with normal uterine and abnormal umbilical artery ratios, group with normal umbilical and abnormal uterine artery ratios, and those in whom both ratios were abnormal, respectively, were 68% ($p < 0.001$), 50% ($p < 0.05$), and 89% ($p < 0.001$).

There were four perinatal deaths during the study, two stillbirths and two neonatal deaths. The mothers of these were all in those in whom both ratios were abnormal.

Nonstress tests were abnormal in 12% of women in group with normal uterine and umbilical artery ratios, 15% in group with normal uterine and abnormal umbilical artery ratios, 11% in group with normal umbilical and abnormal uterine artery ratios, and 78% in those in whom both ratios were abnormal.

Creatinine clearance and fibrinogen levels were also studied. For group with normal uterine and umbilical artery ratios they were (means ± SD) 119 ± 35 ml/min and 485 ± 109 mg/dl; group with normal uterine and abnormal umbilical artery ratios, 85 ± 44 ml/min and 361 ± 121 mg/dl; group with normal umbilical and abnormal uterine artery ratios, 107 ± 30 ml/min and 408 ± 44 mg/dl; and those in whom both ratios were abnormal, 89 ± 34 ml/min and 378 ± 122 mg/dl. None of these values show a difference that is statistically significant.

Table III compares vascular pattern with clinical diagnoses. Forty-three women had chronic hypertension. Sixty-five percent ($p < 0.001$) of them were in the group with normal uterine and umbilical artery ratios, 23% in the group with normal uterine and abnormal umbilical artery ratios, 5% in the group with normal umbilical and abnormal uterine artery ratios, and 7% in those in whom both ratios were abnormal. Thirty-four women had pregnancy-induced hypertension. Fifty-nine percent ($p < 0.001$) were in group with normal uterine and umbilical artery ratios, 15% in group with normal uterine and abnormal umbilical artery ratios, 6% in group with normal umbilical and abnormal uterine artery ratios, and 21% in those in whom both ratios were abnormal. Fifty-one women had preeclampsia. Twenty percent were in the group with normal uterine and umbilical artery ratios, 24% in the group with normal uterine and abnormal umbilical artery ratios, 16% in the group with normal umbilical and abnormal uterine artery ratios, and 41% in those in whom both ratios were abnormal.

Comment

There is a large body of evidence that placental ischemia is present in women whose pregnancies are complicated by preeclampsia.[12] Previous studies have used nitrous oxide diffusion, injection of radioactive sodium into the intervillous space, and placental clearance of labeled dehydroisoandrosterone to show the reduction of uterine blood flow in preeclamptic pregnancies. These techniques have sources of error and frequently require specialized expertise or the injection of radioactive substances. Although they may be valuable research tools, none of these methods are useful to the clinician.

Physiologic states similar to preeclampsia (primarily hypertension and proteinuria) have been produced in a variety of laboratory animals by reducing uterine blood flow.[12] The techniques are difficult and experimental results are frequently not reproducible. No satisfactory animal model of preeclampsia exists today. Whether the reduction of blood flow to the placenta is the cause or effect of preeclampsia remains an open question.

This study demonstrates that the majority of women with clinically significant hypertensive syndromes in pregnancy have evidence of reduced velocity flow in the uterine or umbilical arteries. Women with decreased uterine but normal umbilical flow frequently were delivered of low birth weight infants, but most often this was the result of prematurity rather than growth retardation. There was no significant increase in abnormal nonstress test results or fetal distress in labor. Their disease probably represents a relatively acute event with increased uterine artery resistance and decreased blood flow. This may be a result of vasospasm, thrombosis, or both. Therapeutic regimens aimed at increasing uterine blood flow may be of particular benefit in improving outcome in these pregnancies.

Women with reduced flow velocity in both vessels, uterine and umbilical, have onset of disease earlier in pregnancy and it usually is more severe. We hypothesize that these women never had trophoblastic invasion of the myometrial segment of the spiral arteries that normally occurs at 14 to 16 weeks.[3] To have significant impact on the poor outcome of these pregnancies, intervention will be needed early in pregnancy. More basic research into the mechanisms of implantation are needed. Improving uterine blood flow will probably be more difficult and have less effect on pregnancy outcome than those in the group with normal umbilical and abnormal uterine artery ratios. These women need intensive maternal and fetal surveillance so that delivery can be timed to optimize outcome.

Women with normal uterine but reduced umbilical flow are the most interesting. Although birth weight and gestational age at delivery were similar to those of the group with normal umbilical and abnormal uterine artery ratios, there was a significant increase in the rates of growth retardation and fetal distress in labor. Even in the presence of normal uterine artery development, the umbilical vascular resistance fails to fall as it does in normal pregnancy. This may represent a failure of angioneogenesis because of a lacking fetal factor or acute vasospasm and thrombosis in the placenta. This is a disease state clinically identifiable as preeclampsia that is associated with poor fetal perfusion of the placenta. Based on this, it seems likely that antihypertensive therapy in these patients may have adverse affects on the fetus.

The outcome of pregnancies in women in the group with normal uterine and umbilical artery ratios is not

different from that of the general population. The fetus receives adequate nutrition. Hypertension has little effect on these pregnancies. A disease that clinically appears to be preeclampsia does occur in these women but it occurs late in pregnancy and tends to be mild. Antihypertensive therapy would seem to be of no value to the fetus but may be useful for control of maternal symptoms. Iatrogenic preterm delivery is seldom warranted in these women.

There have been many classifications based on clinical data developed over the years in an attempt to identify those women and their fetuses who are at risk for morbidity and death. Many of these systems are confusing and none are very accurate. The most commonly used system in this country is that prepared by the Committee on Terminology of the American College of Obstetricians and Gynecologists.[18] There are several problems with all classifications. Blood pressure normally varies throughout pregnancy. The absolute value or the change in blood pressure correlates poorly with outcome. Proteinuria, a frequent finding in chronic renal disease, may have little effect on pregnancy outcome. Edema is a frequent normal finding in pregnant women. Classifying women with hypertension according to vascular patterns identifies women with significant disease better than these clinical classifications.

In summary, this study adds additional evidence that placental ischemia occurs in preeclampsia. The ischemia may be present from either the fetal or maternal side. The severity of disease seems to correlate with the degree of ischemia. Those patients with diminished flow velocity from both the fetal and maternal side have poorer outcomes than the other groups.

Doppler velocimetry is a valuable technique that can separate pregnant women with hypertension into four clinically useful categories. A description of the uterine and umbilical artery systolic/diastolic ratio may be helpful in the clinical evaluation of all pregnant women with hypertension. This should lead to better treatment protocols and improved clinical outcome.

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Cocaine use during pregnancy: Adverse perinatal outcome

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Cocaine use has increased dramatically in the United States during the past decade. The life-threatening cardiovascular and central nervous system complications of cocaine have been well documented; however, few studies have examined the risks of cocaine use during pregnancy. In this report the perinatal outcome data of 70 women receiving care at the Perinatal Center for Chemical Dependence of Northwestern University, whose pregnancies were complicated by cocaine abuse, were compared with those of matched control subjects. The use of cocaine during pregnancy was associated with lower gestational age at delivery, an increase in preterm labor and delivery, lower birth weights, and delivery of small for gestational age infants. (Am J Obstet Gynecol 1987;157:686-90.)

Key words: Cocaine, drug abuse, perinatal morbidity

Cocaine abuse has increased dramatically in the United States during the past decade. It is now estimated that 30 million Americans have used cocaine at least once and, of these individuals, 5 million are thought to be using the drug regularly.[1] The reported increase in the use of cocaine by the general population has been paralleled by a similar increase in use among pregnant women.[2] At present, no medically or pharmacologically accepted definitions exist by which the terms "use" and "abuse" may be differentiated. However, the highly addictive nature of cocaine suggests that they may be used interchangeably. The ability of cocaine to precipitate life-threatening cardiovascular and central nervous system complications has been documented in several reports[3-5]; however, few studies to date have examined the risks of cocaine use to maternal and fetal health.[2,6,7] In this report, we analyze 70 pregnancies complicated by abuse of cocaine and other addictive substances and compare these patients with matched obstetric patients without a history of substance abuse.

Material and methods

The Perinatal Center for Chemical Dependence at Northwestern University was established to provide intensive care to patients with pregnancies complicated by substance abuse. This comprehensive program, including obstetric, pediatric, psychiatric, and social services, attempts to eliminate or decrease further substance abuse during pregnancy and postpartum.

From January 1983 to September 1986, 74 women who had used cocaine during their pregnancies received prenatal care commencing by the second trimester in the obstetric clinic of the Perinatal Center for Chemical Dependence. All were delivered of their infants at Prentice Women's Hospital and Maternity Center of Northwestern Memorial Hospital.

Four women were excluded from statistical analysis. These included one woman with lupus anticoagulant and nine previous pregnancy losses who had another intrauterine death, one woman with rhesus sensitization who had three in utero transfusions and was delivered before term, another woman with a history of habitual abortion who had a spontaneous first-trimester abortion, and one woman who had an intrauterine death after a gunshot wound to the abdomen that was sustained while she was using cocaine. The remaining 70 women were delivered of viable singleton infants. Twenty-four (34%) had used only cocaine, 20 (29%) cocaine and marijuana, and 22 (31%) cocaine and opiates (with or without marijuana); the remaining four (6%) had used cocaine and a variety of other illicit substances (amphetamines, phencyclidine, barbiturates, LSD, benzodiazepines).

Control patients were selected from the general obstetric population receiving care at the same institution. Matching was performed on the basis of maternal age, parity, socioeconomic class, tobacco use, and medical complications. All control patients were delivered of their infants within 60 days of the index pregnancy. Study and control pregnancies were compared by use of the appropriate t test for parametric data and either the χ^2 or Fisher's exact test for nonparametric data.

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Table I. Maternal demographic and prenatal data

	Study population (mean ± SD or No. of cases) (n = 70)	*Control population (mean ± SD or No. of cases) (n = 70)*	*Significance (p value)*
Maternal age (yr)	27.0 ± 4.2	27.1 ± 4.2	NS
Gravidity	3.8 ± 2.0	2.7 ± 1.8	<0.0005
Parity	1.1 ± 1.3	1.1 ± 1.1	NS
Prenatal visits	7.9 ± 4.1	10.1 ± 3.8	<0.01
Weight gain (lbs)	27.1 ± 12.0	28.6 ± 11.8	NS
Tobacco use*	29 (41.4%)	19 (27.1%)	NS
Ethanol abuse†	8 (11.4%)	1 (1.4%)	<0.05

*Tobacco use ≥20 cigarettes (1 pack) per day.

†Ethanol use ≥6 drinks per week.

Table II. Obstetric data

	Study population (mean ± SD or No. of cases) (n = 70)	*Control population (mean ± SD or No. of cases) (n = 70)*	*Significance (p value)*
Gestational age at delivery (wk)	37.1 ± 3/6	39.3 ± 2.0	<0.0001
Route of delivery			
Vaginal	55 (78.6%)	54 (77.1%)	NS
Primary cesarean section	8 (11.4%)	10 (14.3%)	NS
Repeat cesarean section	7 (10.0%)	6 (8.6%)	NS
Preterm labor*	15 (21.4%)	1 (1.4%)	<0.001
Preterm delivery*	17 (24.3%)	2 (2.9%)	<0.0005
Birth weight (gm)	2853 ± 698	3382 ± 551	<0.00001
Low birth weight†	16 (22.9%)	3 (4.3%)	<0.005
Small for gestational age‡	13 (18.6%)	2 (2.9%)	<0.001
Abruptio placentae	5 (7.1%)	1 (1.4%)	NS
Congenital anomalies	4§ (5.7%)	1 (1.4%)	NS

*Before 37 weeks' gestation.

†Birth weight <2500 gm.

‡Birth weight less than tenth percentile by birth weight curve of Brenner et al.[8]

§Congenital anomalies include prune-belly syndrome with urethral obstruction (1), amniotic bands with transverse distal limb reduction (1), jejunal atresia and bowel infarction (1), and multiple anomalies including imperforate anus, horseshoe kidney, clubfoot, and amniotic bands (1).

Results

Prenatal data. Maternal demographic and prenatal data for study and control populations are summarized in Table I. The two groups were similar for the matching factors and prenatal weight gain. However, significant differences were present for prenatal visits as well as for concomitant ethanol abuse, reflective of polysubstance abuse. The difference in gravidity between the two populations appeared to be due to an increased number of abortions (spontaneous and elective) in the study patients compared with the control women (1.7 ± 1.5 versus 0.7 ± 1.2, respectively, $p < 0.005$). As information was not available to document cocaine use at the time of previous pregnancy losses, this difference could not be attributed to use of cocaine.

Obstetric data. Table II summarizes comparisons of obstetric and perinatal outcomes. The mean gestational age at delivery was lower in the study patients than in the control women and the proportion of pregnancies delivered before term was higher in the cocaine-using patients than in the control women. Both differences are significant, and these differences may explain the fewer prenatal visits noted in the study patients. The mean birth weight was significantly less in the study group and comparison of the incidence of low birth weight infants (<2500 gm) between the two groups revealed a significantly increased proportion in the study population. The study patients also had a significantly greater proportion of infants with birth weights less than the tenth percentile by the birth weight tables of Brenner et al.[8] when compared with the control women, 13 of 70 or 18.6% versus 3 of 70 or 4.3%, respectively. Furthermore, significantly fewer study patients had infants with birth weights greater than the

Table III. Neonatal data

	Study population (mean ± SD or No. of cases) (n = 70)	*Control population (mean ± SD or No. of cases) (n = 70)*	*Significance*
Sex			
Male	38 (54.3%)	33 (47.1%)	NS
Female	32 (45.7%)	37 (52.9%)	NS
Apgar score*			
1 min	7.3 ± 2.1	7.9 ± 1.5	
5 min	8.6 ± 1.2	9.0 ± 0.5	
Umbilical artery pH	7.26 ± 0.10 (n = 60)	7.25 ± 0.08 (n = 64)	NS
Meconium	20 (28.6%)	16 (22.9%)	NS
Fetal distress/intolerance†	7 (10.0%)	4 (5.7%)	NS

*Infants delivered at ≥37 weeks' gestation.
†Diagnosed on basis of interpretation of the fetal heart rate tracing.

Table IV. Comparison of study subgroups by patterns of abuse

	Cocaine only (n = 24)	*Multiple substances* (n = 46)*	*Significance*
Gestational age at delivery (wk)	36.6 ± 4.2	37.4 ± 3.2	NS
Preterm delivery†	6 (25.0%)	11 (23.9%)	NS
Birth weight (gm)	2677 ± 706	2944 ± 667	NS
Low birth weight‡	8 (33.3%)	8 (17%)	NS
Small for gestational age§	6 (25.0%)	4 (8.7%)	NS
Abruptio placentae	2 (8.3%)	3 (6.5%)	NS
Apgar score			
1 min	6.8 ± 2.5	7.6 ± 1.7	
5 min	8.3 ± 1.7	8.7 ± 0.7	
Umbilical artery pH	7.24 ± 0.14	7.28 ± 0.07	NS

*Cocaine plus any of the following: opiates, marijuana, barbiturates, amphetamines, phencyclidine, benzodiazepines, and LSD.
†Delivery before 37 weeks' gestation.
‡Birth weight <2500 gm.
§Birth weight less than tenth percentile by birth weight curve of Brenner et al.[8]

ninetieth percentile, two of 70 or 2.9% versus 13 of 70 or 18.6% of the control women. The distribution of birth weights in the control women was similar to the distribution in the general obstetric population at this institution when compared by means of the birth weight tables of Brenner et al. The increased frequencies of abruptio placentae and congenital anomalies in the study group (7.1% versus 1.4% and 5.7% versus 1.4%, respectively) compared with the control women suggested clinical if not statistical significance. The increased incidence of both conditions in the study patients suggests the need for close surveillance for these complications in future pregnancies.

Neonatal data. Mean neonatal Apgar scores at both 1 and 5 minutes were lower in the study infants (Table III). However, no differences were observed in the proportion of Apgar scores <7 at either 1 or 5 minutes. Comparison of umbilical artery pH values showed no significant difference in mean values or in the frequencies of pH values either <7.20 or <7.10. Similarly, there were no differences in the frequencies of either meconium or fetal distress between the two groups.

In an attempt to identify subgroups of the study population with increased morbidity, patients who had used only cocaine were compared with those abusing a variety of addictive substances in addition to cocaine (Table IV). The mean birth weights were lower in the cocaine only group, and the proportions of pregnancies with low birth weight (<2500 gm) and birth weights less than the tenth percentile were higher in this group than in the study population who abused multiple substances; however, these differences were not statistically significant.

Comment

The true extent of cocaine use during pregnancy is difficult to estimate, and the maternal and fetal risks of cocaine use have not yet been well documented. Chasnoff et al.,[2] in a report including 23 cocaine-abusing women, suggested that cocaine use during

pregnancy is associated with an increase in previous spontaneous abortions, abruptio placentae, congenital anomalies, and abnormal Brazelton Neonatal Behavioral Assessment Scale evaluations. In another report of only eight patients, Madden et al.[7] concluded that there was no evident symptomatology in the infants born to cocaine-abusing women and suggested that special management schemes were not needed for these infants. However, examination of their data reveals that two (25%) of these infants were assessed as being small for gestational age and one (12.5%) of the infants was delivered before term.

The cocaine-using pregnant woman and her fetus are at risk for life-threatening cardiovascular and central nervous system complications.[3-5] In addition, they are at risk of other potential hazards that are specific to cocaine use during gestation. Among these are the following: decreased uteroplacental blood flow and uteroplacental insufficiency (acute and chronic), maternal hypertension, stimulation of uterine contractions, and fetal vasoconstriction.[9, 10] Uteroplacental insufficiency is associated with diminished birth weight, intrauterine growth retardation, and fetal intolerance to labor.[11] Indeed, infants of women using cocaine had significantly decreased birth weight and an increased incidence of small for gestational age infants (Table II). Maternal hypertension, especially when associated with alterations in uteroplacental blood flow, may increase the risks of abruptio placentae. Although our data failed to reveal statistically significant differences in the frequencies of abruptio placentae between study and control groups, clinically this difference is alarming. If indeed larger studies in the future confirm an association between cocaine use and abruptio placentae, this association could have a significant impact on maternal and fetal morbidity and mortality. Fetal vascular compromise has been suggested as the cause of congenital anomalies, in particular, limb reduction defects,[12] and while our study failed to reveal a significant difference in the incidence of congenital anomalies between the two groups, larger studies may also be necessary to examine this relationship.

The present study data suggest that cocaine use indeed may adversely affect perinatal outcome. Specifically, the use of cocaine during pregnancy was associated with lower gestational age at delivery, an increase in preterm labor and delivery, lower birth weights, and delivery of small for gestational age infants. At present, these data can only be interpreted as demonstrating an association, rather than a causal relationship, between cocaine use during pregnancy and perinatal morbidity because the fundamental pharmacologic data on the effects of cocaine in human pregnancy are incomplete.

It is important to acknowledge several factors that may have influenced our results. First, the cocaine abuser has a marked tendency to abuse more than one substance.[1] As previously noted, only 34% of the study population used cocaine alone. Our inability to show differences on the basis of patterns of substance abuse (Tables IV) suggests that it may matter little if cocaine is used either alone or with other substances of abuse. Analysis of data relative to pattern of abuse based on trimester use was not possible since random urine toxicology screens were available for only the 38 most recent patients. Patient recall cannot be considered reliable to assess trimester use. In the future, pregnancies will be analyzed by use of urine toxicology screens to evaluate the influence of trimester abuse patterns and therapeutic interventions on perinatal outcome. In the patients that have random screening data, only 33% have eliminated cocaine abuse during pregnancy, which was significantly less than the success of therapeutic interventions when cocaine was not the substance of abuse. Information to reliably assess the quantity of cocaine used by each study patient or the purity of the cocaine was also not available. It is recognized that the latter factor may be highly variable.[1]

Second, tobacco use and poor nutritional intake are recognized as being associated with diminished fetal growth.[13, 14] In this study, however, study and control patients were matched for tobacco use, and although detailed dietary histories were not available, no significant differences in prenatal weight gain were noted between the two groups (Table II).

Third, it is possible that our study population may represent only a select portion of women using cocaine during gestation. If indeed cocaine has an adverse effect on pregnancy outcome, as suggested by these study data, selection of patients with the worst patterns of abuse may influence study findings. However, it is also possible that this group may indeed have a more favorable outcome than those patients with similar cocaine abuse that receive no prenatal care.

Finally, despite this being the largest study of cocaine pregnancies to date, the total number of patients studied is limited. It is perhaps more important that the life-style of the addicted pregnant woman simply is not comparable to that of the nonaddicted pregnant woman for the following reasons: (1) As the addiction worsens, the need to procure larger quantities of the addictive agent and the need for more frequent use overwhelms all other considerations of maternal health and fetal well-being; (2) the financial requirements of a heavy addiction are ruinous regardless of the income status of the addict; (3) behavioral patterns commonly considered unacceptable by society frequently emerge as the addict struggles to support her addiction; (4) disintegration of the premorbid personality is a frequent sequela to the exhaustion of physical, emotional, and financial resources.

In spite of the potential limitations of our data, they suggest that cocaine use is associated with preterm delivery, lower birth weight, and small for gestational age infants. Although preterm delivery accounts for only 6% to 7% of all deliveries, 75% of perinatal morbidity and mortality occurs in preterm infants.[15] It is thus conceivable that widespread cocaine use during pregnancy could become a significant and continuing factor adversely affecting local, regional, and national perinatal statistics.

Because of the well-documented vasoconstrictive properties of cocaine,[10] it is also plausible to suggest that cocaine may have a causal relationship to the increased morbidity observed in our study population. Further research is necessary to investigate the effects of cocaine in pregnancy, in particular, its placental and fetal sympathomimetic actions, and to clarify the precise nature of the effects of cocaine in laboratory and larger population studies.

At present we are implementing a policy of more aggressive antenatal surveillance in our clinics, including ultrasound examinations for assessment of congenital anomalies and fetal growth, serial fetal nonstress testing, umbilical Doppler flow studies, and fetal neurobehavioral evaluation. However, education programs aimed at prevention of the initiation of cocaine use are more desirable. To date, the dangers of cocaine have not been adequately recognized by the general public or their physicians.[16]

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Neonatal morphometrics and perinatal outcome: Who is growth retarded?

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To evaluate the relationship between neonatal morphometrics and poor neonatal outcome resulting from fetal malnutrition, we prospectively examined 355 sequential live-born, singleton neonates >35 weeks' gestational age. Birth weight, neonatal ponderal index, and midarm circumference to head circumference ratio were measured. A birth weight, ponderal index, or midarm circumference to head circumference ratio ≤tenth or ≥nintieth percentiles for gestational age was considered abnormal. Poor outcome was defined as operative delivery for fetal distress, 5-minute Apgar score <7, meconium aspiration, polycythemia, or hypoglycemia. Thirty-three (9.3%) neonates had perinatal morbidity. Although morbidity was not increased among neonates that were large by any of the criteria, five (25%) of 20 with low birth weight had poor outcome and 18 (26%) of 70 with low ponderal index or midarm circumference to head circumference ratio suffered morbidity. Of the 33 neonates with morbidity, 18 (54.5%) had low ponderal index or midarm circumference to head circumference ratio, but only five (15.2%) had low birth weight. Therefore, low ponderal index and midarm circumference to head circumference ratio are more sensitive predictors of outcome than is birth weight. As such, ponderal index and midarm circumference to head circumference ratio are more appropriate end points for antenatal diagnostic studies than is birth weight. (AM J OBSTET GYNECOL 1987;157:691-3.)

Key words: Neonatal morphometrics, intrauterine nutrition, perinatal outcome

The relationship between birth weight and perinatal outcome is well described, and classification of neonates as small, appropriate, or large for gestational age is common practice.[1-3] Despite the fact that such classification implies only proximity to a mean weight, small infants are commonly referred to as "growth retarded." If neonates are classified by birth weight for gestational age only, many growth-retarded neonates are nutritionally normal and are at no increased perinatal risk.[4]

Recognizing the heterogeneous pathophysiology of variations in fetal growth, some authors recommend neonatal classification based on nutritional status rather than birth weight.[5-7] Ponderal index and midarm circumference to head circumference ratio should theoretically differentiate the malnourished neonate, who is likely to experience perinatal morbidity and death, from the small, normal neonate.

Knowledge of the neonatal morphometrics most commonly associated with poor perinatal outcome is important to perinatologists. Such insight will enable us to develop antenatal diagnostic tests to identify equivalent fetal characteristics and, hopefully, decrease perinatal morbidity. Assuming that abnormalities in fetal growth often result in poor perinatal outcome, we prospectively studied the value of birth weight percentile, ponderal index, and midarm circumference to head circumference ratio in predicting the occurrence of perinatal morbidity. The method most closely related to outcome should provide the most useful prognostic information and should be the end point of antenatal diagnostic endeavors.

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Method

At Medical Center Hospital from August 1 to October 31, 1986, a prospective, random, sequential sample of singleton live-born, normally formed infants delivered after 35 weeks' gestational age was selected for study. Weight and crown-heel length were recorded immediately after birth. Ponderal index was calculated ([weight in gm./(length in cm)^3] × 100). Twenty-four to 36 hours after delivery, each neonate was examined by trained personnel and an occipitofrontal head circumference and Dubowitz score were recorded. Gestational age was established on the basis of obstetric data and confirming Dubowitz. The arm circumference at midhumerus was measured with a tape bilaterally and the two measurements were averaged. Midarm circumference to head circumference ratio was calculated ([midarm circumference to head circumference ratio] × 100). Abnormal birth weight, ponderal index or midarm circumference to head circumference ratio was

Table I. Perinatal outcome among 355 neonates classified by birth weight and nutritional status

Perinatal outcome	*Birth weight percentile*					
	≥Nintieth percentile (n = 49)		*<Nintieth percentile and >tenth percentile (n = 286)*		*≤Tenth percentile (n = 20)*	
	Normal	*Thin*	*Normal*	*Thin*	*Normal*	*Thin*
Good (n = 322)	41	2	220	44	9	6
Poor (n = 33)	5	1	10	12	0	5
p Value	0.33		<0.001		<0.03	

Thin = Low ponderal index or low midarm circumference-to-head circumference ratio.

defined as values ≥nintieth or ≤tenth percentiles for gestational age.[2, 5, 7]

An infant with a normal ponderal index and midarm circumference to head circumference ratio was considered well-nourished, whereas an infant with either a low ponderal index or a low midarm circumference to head circumference ratio was considered thin.

Perinatal morbidity was defined as operative delivery for fetal distress, 5-minute Apgar score <7, meconium aspiration, polycythemia, or hypoglycemia.

Statistical analysis was performed by χ^2 or Fisher's exact test where appropriate. A value of $p \leq 0.05$ was considered significant.

Results

A total of 355 patients were enrolled in the study. The mean gestational age and birth weight were 39.3 ± 1.3 weeks and 3314 ± 454 gm, respectively. The mean ponderal index was 2.56 ± 0.3 and the mean midarm circumference to head circumference ratio was 27.2 ± 3.0. Thirty-three (9.3%) patients had perinatal morbidity. There were no neonatal deaths.

Birthweight, ponderal index, and midarm circumference to head circumference ratio were significantly related to the occurrence of perinatal morbidity. However, morbidity was more strongly related to ponderal index ($p < 0.004$) and midarm circumference to head circumference ratio ($p < 0.001$) than to birth weight ($p < 0.03$). Classification of a neonate as large by any of the three methods was not associated with increased perinatal morbidity ($p > 0.43$). Of 20 patients with low birth weight, five (25%) suffered morbidity. Morbidity occurred in 10 (23%) of 44 neonates with low ponderal index and in 11 (36%) of 31 with low midarm circumference to head circumference ratio.

Twenty patients had low birth weights for gestational age. Eleven (55%) were thin. All five patients who suffered perinatal morbidity in this group were thin. Conversely, in all nine well-nourished but lightweight infants, outcome was good ($p = 0.03$) (Table I).

Birth weight was normal in 286 patients. Twenty-two (7.7%) suffered perinatal morbidity. There were 230 well-nourished neonates, 10 (4.4%) of whom suffered morbidity. In contrast, morbidity occurred in 12 (21.4%) of 56 thin neonates with normal birth weight. Of the 22 patients with perinatal morbidity, 12 (54.6%) were thin (Table I).

The occurrence of perinatal morbidity was not significantly related to nutritional status among the 49 neonates with high birth weights.

Among the 355 patients, 70 (19.7%) were thin. Eighteen (25.7%) of those suffered morbidity. Of the 285 well-nourished neonates, 94.7% had normal outcome. With ponderal index and midarm circumference to head circumference ratio used to evaluate neonatal nutritional status, 18 (54.5%) thin neonates were identified among the 33 with morbidity, whereas only five (15.2%) of the 33 had low birth weight for gestational age (Table I).

Seven neonates had both low ponderal index and low midarm circumference to head circumference ratio. Five (71%) of those suffered morbidity. Only one of the five with morbidity had low birth weight.

Birth weight and nutritional status were normal in 276 neonates, 15 (5%) of whom suffered morbidity. Among 59 thin subjects with normal birth weight, 13 (22%) had morbidity. Poor outcome occurred among five of 11 (46%) thin neonates with low birth weight, but there was no morbidity among the nine who were small but well-nourished (Fig. 1).

Comment

Growth retardation is commonly defined as low birth weight for gestational age. However, many small neonates are normal, and one need only recall a neonate with postmaturity syndrome to realize that fetal malnutrition is often independent of birth weight. Scott and Usher[8] demonstrated that small, clinically well-nourished neonates rarely suffered peripartum asphyxia, whereas those with soft-tissue wasting commonly had poor outcome. In another study, Hill et al.[6] found that, regardless of birth weight, long-term neurologic morbidity was more frequent among patients with soft-tissue wasting at birth.

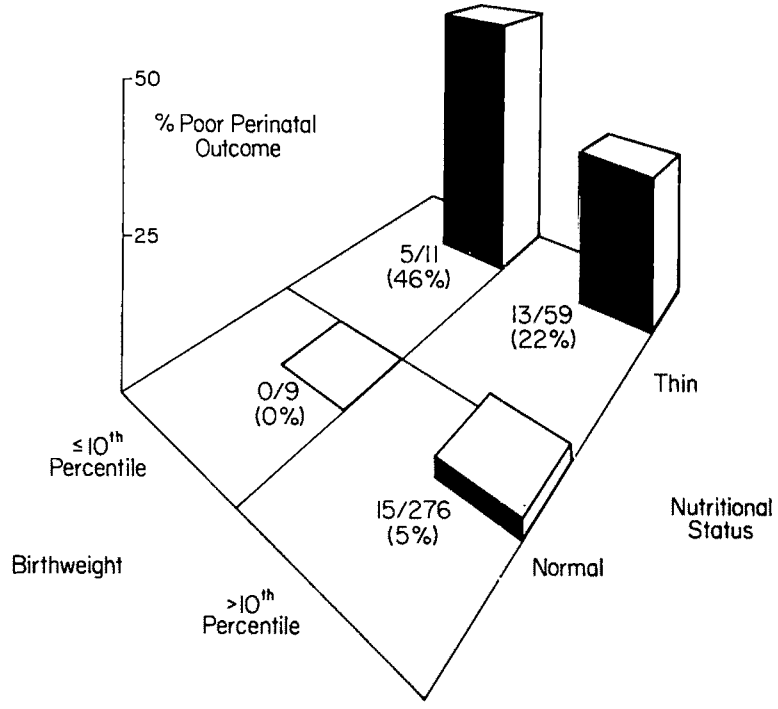


Fig. 1. Outcome among neonates classified by birth weight and nutritional status. Thin = low ponderal index or low midarm circumference to head circumference ratio.

The hypothesis of this study was that nutritional status is more important prognostically than is birth weight. The relationship of birth weight, ponderal index, and midarm circumference to head circumference ratio to perinatal outcome has been documented previously.[1,3-5,7] In this study, classification of each neonate by three different methods allowed for parallel analysis of the relevance of each method. Such an analysis allowed us to identify the method that provided the most useful prognostic information in terms of perinatal morbidity.

The data demonstrate that being small or thin places the fetus and neonate at greater risk for morbidity. Of 20 small neonates, five (23.8%) suffered morbidity. Eighteen of 70 (25.7%) thin neonates had morbidity. However, of the 33 neonates with perinatal morbidity, only five (15.2%) were small, whereas 18 (54.5%) were thin. Therefore, ponderal index and midarm circumference to head circumference ratio were much more sensitive prognosticators than was birth weight.

In conclusion, assessment of nutritional status helps to identify neonates at increased risk for perinatal morbidity, regardless of birth weight. Current methods of surveillance of pregnancies at risk for fetal malnutrition are primarily designed to predict low birth weight.[9] However, these data demonstrate that predicting a thin neonate is more meaningful than predicting birth weight. These findings must be given due consideration in designing antenatal diagnostic studies.

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The nonpredictive value of fetal urinary electrolytes: Preliminary report of outcomes and correlations with pathologic diagnosis

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Fetal urine was sampled 12 times in nine fetuses with sonographically diagnosed urinary tract obstruction to assess renal function. By previously proposed criteria, four fetuses were predicted to have poor renal function. Two of these fetuses were found to have renal dysplasia on autopsy after elective termination. The other two died in the neonatal period but only one of these had histologic evidence of renal dysplasia. Five fetuses were predicted to have good renal function. Three of these developed renal failure after birth, one was found to have renal dysplasia on autopsy after elective termination, and one is alive and well. We conclude that fetal urine electrolytes are not necessarily an accurate predictor of neonatal renal function. (AM J OBSTET GYNECOL 1987;157:694-8.)

Key words: Fetal hydronephrosis, urine electrolytes, fetal renal function

Fetal urinary tract dilatation detected by ultrasonography is a finding associated with a spectrum of prognoses. Mild hydronephrosis in utero may disappear or remain stable, and if it is not progressive it probably has no adverse consequences for the neonate. However, more severe degrees of dilatation can reflect partial or complete urinary tract obstruction that may lead to neonatal renal failure and, if severe oligohydramnios is present, may be associated with pulmonary hypoplasia.

Deciding where a particular case falls in this spectrum is often a challenging task. Clearly, an accurate assessment of renal function and the extent to which it can be expected to deteriorate would be invaluable when candidates are selected for invasive in utero therapy as opposed to expectant management.[1-3] Various predictors of fetal outcome based on ultrasonographic findings or attempts to quantitate urine output in utero have been proposed, but none are entirely reliable. One recently proposed method for evaluating the functional status of an obstructed fetal genitourinary tract is to analyze the fetal urinary electrolytes and osmolarity.[2] The purpose of this study was to prospectively assess these parameters of renal function when managing fetuses with ultrasonic evidence of severe urinary tract obstruction.

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Table I. Prognostic criteria for urinary electrolytes

Prognosis	*Sodium (mEq/ml)*	*Chloride (mEq/ml)*	*Osmolarity (mosm)*
Good	<100	<90	<210
Poor	>100	>90	>210

Modified from Golbus et al.[2]

Methods

During a 2-year period, nine patients who have subsequently been delivered were referred to the Mount Sinai Medical Center for consideration of invasive therapy in utero because of an obstructive fetal uropathy. In each case a complete ultrasonographic examination was carried out to characterize the obstruction and rule out associated anomalies. In addition to performing a thorough anatomic survey of the fetus, this examination included a search for renal cysts, hydronephrosis and hydroureters, quantification of bladder size and all loculated collections of urine, and a subjective assessment of amniotic fluid volume. In defining amniotic fluid volume, measurements of individual pockets were not used. Our subjective criteria were as follows: Severe oligohydramnios was present when no amniotic fluid was seen in the uterus. Mild oligohydramnios was present when the volume of fluid was somewhat less than expected for the gestational age. Moderate oligohydramnios included all other cases of decreased fluid. Chromosomal analysis was attempted in all but one case. If the pregnancy was early enough the option of elective termination was given to the parents.

Table II. Urinary electrolytes obtained and predicted results

Patient No.	*Sodium (mEq/ml)*	*Chloride (mEq/ml)*	*Osmolarity (mosm)*	*Predicted function*
1	146	117	283	Poor
2	59	57	N/A	Good
	62	56	N/A	Good
3	110	93	245	Poor
4	73	55	154	Good
	69	58	148	Good
5	62	55	137	Good
6	71	57	153	Good
	68	62	158	Good
7	113	118	261	Poor
8	130	100	265	Poor
9	76	67	161	Good

N/A = Not available.

In conjunction with the ultrasonographic evaluation, each patient underwent at least one aspirate of fetal urine for analysis. This was performed by sterile technique under direct ultrasound visualization by introducing a 20- or 22-gauge spinal needle into the fetal bladder or dilated renal pelvis. The urine was tested for sodium, potassium, and chloride concentrations and osmolarity. These results were interpreted according to previously published guidelines[2] (Table I) and were used to counsel patients about termination, if appropriate, or further invasive procedures.

Results

Nine fetuses underwent urine sampling. The ultrasonographic findings in each of these cases along with urine values and outcome are listed in Tables II and III. Three pregnancies were terminated electively and the other six were delivered between 29 and 37 weeks.

Antenatally these cases were classified by the diagnosis made from the ultrasonographic findings. Five fetuses had obstruction consistent with a posterior urethral valve or lower tract obstruction. In each case an enlarged bladder was seen, and in four of these cases bilateral hydronephrosis was also observed. Two fetuses had findings suggestive of ureterovesical junction obstruction, unilateral in one case and bilateral in the other. In these two fetuses, bladder size was normal, but hydroureter and hydronephrosis were seen. The remaining two fetuses had ureteropelvic junction obstruction, which was unilateral in one case and bilateral in the other. In these cases isolated hydronephrosis was seen.

Amniotic fluid volume was variable, ranging from severe oligohydramnios to polyhydramnios. Although no fetus with severe oligohydramnios survived, one of these infants who died of pulmonary hypoplasia did not have renal dysplasia detected on autopsy. Otherwise, the volume of amniotic fluid did not correlate with outcome.

Chromosome studies were attempted in eight fetuses, and there was one failed culture. Of the remaining seven fetuses, one had Klinefelter's syndrome (47,XXY) and the others were normal males (46, XY). The fetus with Klinefelter's syndrome also had a ventricular septal defect. No other fetus had any associated anomalies. Chromosomes were obtained from amniotic fluid in four cases, fetal blood in two cases, and fetal urine in two cases.

The electrolytes and osmolarity of all the fetal urinary aspirates are shown in Table II. A second sampling was performed in three fetuses (10, 18, and 57 days after the first), and in each case no significant change was noted in the values in the second specimen.

In four fetuses urinary electrolyte values were consistent with poor renal function. Three of these had ultrasonographic findings of posterior urethral valve and one had unilateral ureteropelvic junction obstruction. The latter fetus (No. 8) had a mildly increased volume of amniotic fluid. In this fetus the urine was aspirated from the dilated renal pelvis. Because the other kidney, bladder, and amniotic fluid volume appeared to be normal, the patient was given a favorable prognosis but elected to terminate the pregnancy. At autopsy, bilateral renal dysplasia was detected.

Only one of the remaining three fetuses with posterior urethral valve and poor electrolytes presented in the second trimester (No. 7). After elective termination the prune belly syndrome with bilateral renal dysplasia was diagnosed at autopsy. The remaining two fetuses (Nos. 1 and 3) were sampled at 27 and 28 weeks' gestation. Although both of these fetuses died shortly after delivery of pulmonary hypoplasia, only one was found to have renal dysplasia at autopsy. The other fetus (No. 3) had histologically normal kidneys with hydronephrosis. No urethral stricture was found. This fetus also had Klinefelter's syndrome and a ventricular septal defect.

In the other five fetuses urinary electrolytes were within the normal range when sampled. Of these, two

Table III. Ultrasound findings, treatment, and outcomes

Patient No.	*Ultrasonographic findings*					*Gestational age at diagnosis (wk)*
	Hydronephrosis	*Hydroureter*	*Large bladder*	*Kidneys*	*Oligohydramnios*	
1	+	+	+	Normal	Severe	28
2	+	+	−	Normal	Moderate	28
3	+	−	+	Normal	Severe	27
4	−	−	+	Echolucent areas	Moderate	27
5	+	−	−	Normal	Mild	18
6	+	+	+	Normal	None	18
7	+	+	+	Normal	Severe	17
8	+*	−	−	Cystic areas	Polyhydramnios	20
9	+*	+	−	Cystic areas	None	30

*Unilateral.

had a posterior urethral valve, two had ureterovesical junction obstruction, and one had bilateral ureteropelvic junction obstruction. The latter fetus (No. 5) had an elective termination and bilateral renal dysplasia at autopsy.

The two fetuses with posterior urethral valve are the two survivors in this series. In one fetus (No. 4) a vesicoamniotic shunt was placed at 30 weeks' gestation and seemed to malfunction at 33 weeks, at which time the fetus was delivered. This infant is doing well. The other fetus (No. 6) was first evaluated at 18 weeks' gestation. The parents chose not to terminate the pregnancy after having been given a good prognosis after urine sampling at 24 weeks. An attempt was made to place a vesicoamniotic shunt at 32 weeks because of the recent appearance of oligohydramnios, but the attempt failed and the infant was delivered. At the time of the attempted shunt placement, repeat electrolytes were obtained that were unchanged from 8 weeks previously. The neonate had a rising serum creatinine level from birth despite relief of the obstruction. A renal biopsy performed in the neonate revealed a large area of dysplasia. The infant is alive at 7 months of age with moderate renal insufficiency and is awaiting transplant.

One of the two fetuses with ureterovesical obstruction (No. 9) was thought to have unilateral disease on ultrasonographic examination. The other kidney appeared to be normal as did the amniotic fluid volume. The bladder was top normal size. Electrolytes obtained from a bladder aspiration at 31 weeks were normal and the patient was followed expectantly. At 37 weeks' gestation, for the first time, mild oligohydramnios became apparent. After delivery the infant had a rapid downhill course and died at age 7 weeks while on peritoneal dialysis. Autopsy revealed bilateral renal dysplasia, unilateral hydronephrosis, and prune belly syndrome.

The second fetus with ureterovesical obstruction (No. 2) had bilateral disease and was delivered at 31 weeks' gestation after corticosteroid administration. Electrolytes at 28 and 30 weeks' gestation were well within the normal range. The neonate developed progressive renal failure and died at 6 weeks of age. The prune belly syndrome, bilateral renal dysplasia, and a tight urethral stricture were demonstrated at autopsy.

Comment

When urinary tract obstruction is detected in utero, options for management include early delivery, expectant management, and the performance of invasive in utero procedures. Assessment of renal function should be a critical variable in determining which option is appropriate for any given fetus. If renal function is poor, aggressive therapy is not warranted because irreversible damage is likely to have occurred, whereas if good renal function is documented options should be considered that will minimize ongoing damage to the kidneys.

Ultrasonography is the most useful method available for the initial assessment of these disorders. Of primary importance is the differentiation between obstructive and nonobstructive lesions whenever possible. Although this may be suggested by the pattern of dilatation, this distinction cannot always be made.[4, 5] This difference is important because fetuses with nonobstructive lesions are not candidates for shunt placement, and without therapy these neonates usually do very well.

Another vital component of the initial ultrasonographic examination in these patients is the search for associated anomalies. The detection of additional anomalies should disqualify these fetuses from being candidates for invasive therapy.[6] Some controversy ex-

Gestational age when sampled (wk)	*In utero treatment*	*Gestational age at delivery (wk)*	*Predicted function*	*Outcome*
28	Shunt placement	29	Poor	Died age 14 hr; renal dysplasia
28 + 30	None	31	Good	Died age 6 wk; renal failure
27	None	32	Poor	Died age 12 hr; no dysplasia
28 + 30	Shunt placement	33	Good	Alive and well age 18 mo
20	None	20	Good	Elective termination; renal dysplasia
24 + 32	Attempted shunt placement	33	Good	Alive age 7 mo with renal failure
17	None	17	Poor	Elective termination; renal dysplasia
22	None	23	Poor	Elective termination; bilateral renal dysplasia
31	None	37	Good	Died age 7 wk; renal failure, severe dysplasia

ists as to the frequency of associated anomalies. Nicolaides et al.[7] described a 23% rate of chromosomal abnormality in their series of 39 fetuses with obstructive uropathy, although this is a higher frequency than is usually quoted. Quinlan et al.[5] found a lower rate of chromosomal abnormalities, only one abnormal karyotype in nine fetuses tested, but 42% had associated anomalies in other systems.

If the ultrasonographic findings are compatible with urinary tract obstruction in the absence of associated anomalies or an underlying chromosomal aneuploidy, a careful selection must be made for candidates for aggressive therapy. Ideal cases are those fetuses at risk for serious renal compromise who have good renal function at the time of diagnosis and no other detectable anomalies.

At this time no completely reliable antepartum method exists for predicting renal function in the neonatal period. Several techniques have been suggested as predictors including assessment of amniotic fluid volume, ultrasonographic evaluation of the renal parenchyma, quantification of upper tract dilation, and analysis of various constituents of fetal urine.[2] Of these, urinary electrolyte and osmolarity analysis has appeared to be the most promising.

Data derived retrospectively from the patients followed by Golbus et al.[2] have suggested that fetuses with isothenuria do poorly, whereas fetuses with dilute urine do well. The cutoff values proposed from this analysis include a sodium level <100 mEq/ml, a chloride level <90 mEq/ml, and an osmolarity value <210 mOsm.

In theory this is an attractive model. It suggests that nonfunctioning kidneys act like a sieve with respect to filtration of serum. On the other hand, functioning kidneys display the normal, selective tubular reabsorption of sodium and chloride. The fetuses in our series were managed prospectively according to these proposed criteria to judge degree of renal impairment in utero. Because the urine sampled in most cases was from the bladder, which is stagnant, this may not accurately reflect the urine that is simultaneously being excreted by the kidney.

In this series, although there were no survivors among the four fetuses with poor predicted function, one had no evidence of renal disease at autopsy. However, the other three were documented to have dysplastic kidneys.

On the other hand, urinary values below the abnormal cutoffs did not reliably exclude the finding of dysplasia or evidence of severe renal dysfunction during the neonatal period. All fetuses with normal values had minimal degrees of oligohydramnios or normal amniotic fluid volumes and the two surviving infants in this series are in this group.

In the neonate, as in the fetus, it may be difficult to assess renal function. Because the serum creatinine level does not begin to rise until after birth, for the first few weeks of life it may not be possible to use this index. Similarly, although urine output can be measured directly, this may be misleading because of postobstructive diuresis in cases in which the sites of obstruction have been bypassed or because of concomitant problems of prematurity or incomplete relief of obstruction. In these situations urinary electrolytes, particularly sodium values, have been suggested as markers of renal function but have been unreliable.[8]

Although our results are seemingly at variance with the findings of Golbus et al.,[2] we have used this information to predict function at a future date, something he did not propose. It may be argued that these urinary parameters are only a reflection of the current status

of the fetus and that a delay in delivery invalidated their use in these cases. However, in those cases in which predicted function via electrolyte analysis did not correlate with outcome at delivery, a delay in delivery alone was not an adequate explanation for the discrepancy.

In patients 2, 5, and 6, <1 week passed between the final fetal urine analysis and delivery. A 5-week delay in delivery followed sampling in patient 3; however, because the values were predictive of dysplasia and it did not exist at 32 weeks, it could not have been present at 27 weeks. Finally, in patient 9 there was a 6-week delay from urinary sampling to delivery. The postmortem histologic findings of severe dysplasia imply an early profound insult that is not the result of progressive disease in the third trimester. Finally, even if this test is only predictive of current renal status, this will weaken its value as an indicator of neonatal outcome and thus its value as an adjuvant to counseling patients with a fetus with obstruction.

In this group of patients three different outcome variables have been used: neonatal renal function, death, and pathologic diagnosis. Although neonatal renal function was our ultimate concern, it was not measurable in three electively terminated fetuses or two neonates who died shortly after birth. Using death alone as an outcome parameter is problematic because other nonrenal causes of neonatal death such as pulmonary hypoplasia are included, whereas neonates who survive with renal impairment are not. In addition, electively terminated fetuses could not be included in such an analysis. Thus the pathologic diagnosis of dysplasia is an important end point. In all cases of dysplasia found at autopsy the damage was so severe and extensive that, in the opinion of our pathologists, it was not compatible with normal renal function.

There remain many unanswered questions in the prenatal management of fetal urinary tract obstruction. First, there is controversy about whether relieving severe obstruction is useful when this is present early in gestation. Seeds and Mandell[9] have suggested that intervention may not be indicated in these cases because renal damage is uniformly too advanced, but this has been disputed by Manning et al.[10] Conversely, when good renal function is present in the third trimester, it may be unlikely that relief of obstruction offers any advantage over expectant management and early delivery.[4, 11]

Finally, in all of these cases renal failure in the child who is apparently normal at birth may develop during the first few years of life. Thus, long-term follow-up of reported cases must also be sought to fully evaluate the success of intervention.

In conclusion, although assessment of fetal renal status in cases of obstructive uropathy in utero is of great potential value, this study suggests that assessment of fetal urinary electrolytes and osmolarity is not an optimal way to accomplish this goal. In four cases in which the values suggested poor function the outcome was universally dismal, but one of these fetuses did not have evidence of dysplasia at autopsy. On the other hand, in five cases in which fetal urinary studies predicted good renal function, a wide spectrum of findings were observed in the neonates.

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Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short- and long-term metabolic control in gestational diabetes

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Glycosylated hemoglobin and glycosylated serum protein have been suggested as tools for evaluation of long- and short-term glycemic control, respectively. Twenty-six patients with gestational diabetes were prospectively studied to determine the relationship of glycosylated hemoglobin and glycosylated serum protein to metabolic control. To verify the accuracy of blood glucose data, a memory-based reflectance meter was used for subjects with gestational diabetes who tested 6.5 ± 1 times per day. Our analysis revealed that despite a statistically positive correlation between glycosylated hemoglobin, glycosylated serum protein, and verified data, their use as a clinical tool is limited because of their poor predictability. (Am J Obstet Gynecol 1987;157:699-703.)

Key words: HbA_1, glycosylated serum protein, verified blood glucose data

The association between the level of metabolic control and perinatal complication of diabetes in pregnancy is well established.[1,2] Therefore, methods to evaluate glycemic control in these patients have been a subject of research during the past several years. It has been suggested that glycosylated hemoglobin (HbA_1) and glycosylated serum protein are useful as clinical tools in assessing long- and short-term glycemic control, respectively.[3,4] Several investigators have found that the level of HbA_1 in pregnant patients is directly related to blood glucose values and reflects glucose control for the previous 4 to 12 weeks (long-term control), paralleling the life span of the red blood cell.[5-7] In contrast, glycosylated serum protein was found to be a predictor of short-term glycemic control (1 to 2 weeks).[3] Our study set out to determine the relationship between HbA_1, glycosylated serum protein, and verified self-monitored blood glucose.

Method and material

A prospective study involving 26 gestational diabetic and 18 nondiabetic women was performed. Subjects with hemoglobinopathies, renal failure, and a history of drug abuse were excluded. Gestational diabetes was diagnosed pursuant to the National Diabetic Data Group criteria.[8] Obesity was measured by body mass index (kilograms/height^2), using prepregnancy weight. From the time of diagnosis of gestational diabetes, patients were given a memory-based reflectance meter assuring reliable documentation of blood glucose data.[9] Capillary blood glucose was to be tested seven times a day, which included fasting, preprandial, 2-hour postprandial, and bedtime values from the time of diagnosis to delivery. Patients were instructed in the use of the memory-based reflectance meter and tested for their skills at entry to the study and each subsequent visit. Patients were seen at two-week intervals. During each clinic visit blood samples for HbA_1 and glycosylated serum protein were drawn. All samples were obtained and analyzed during the same day in the General Clinical Research Center Laboratory. HbA_1 was measured by a microcolumn cation exchange method with the normal range defined as between 5% and 7%. Glycosylated serum protein was evaluated with the Δ Glyc-Affin Glycosylated Serum Protein assay in which glycosylated serum protein was separated by a chromatographic column and then its total percent calculated by a spectrometer evaluation at 595 mm. Normal values of glycosylated serum protein were established at 8% to 12.9%.

For the controls, one HbA_1 and glycosylated serum protein determination after a normal glucose tolerance

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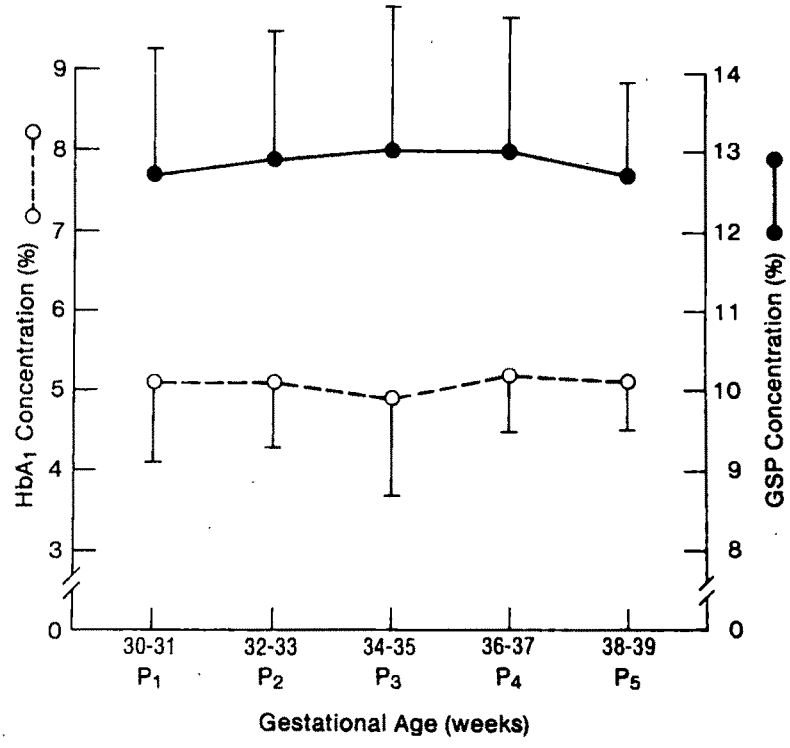


Fig. 1. Longitudinal assessment of glycosylated serum protein *(GSP)* and HbA_1 in pregnancy (mean ± SD). The solid line with closed circles indicates the mean values of GSP in relationship to time periods of gestational age. The dashed line with open circles represents the association of HbA_1.

Table I. Patient characteristics

	GDM	*Control*
Age (yr)	29 ± 6	27 ± 5
Parity (%)		
Nulliparity	50	62
Multiparity	50	38
Obesity (BMI 27.3) (%)	68	26
Time of diagnosis (wk gestation)	29 ± 4	29 ± 2
Time of delivery (wk gestation)	39 ± 3	39 ± 2
Treatment (%)		
Insulin	58	—
Diet	42	—

GDM, gestational diabetes; BMI, body mass index.

test result was performed to establish the normal range for both assays in pregnancy. For subjects with gestational diabetes, analysis was performed to evaluate HbA_1 and glycosylated serum protein with respect to blood glucose data for five periods: period 1, weeks 1 and 2; period 2, weeks 3 and 4; period 3, weeks 5 and 6; period 4, weeks 7 and 8; and period 5, weeks 9 and 10. Additional analysis was performed on blood glucose data cumulative for 4, 6, 8, and 10 weeks compared with HbA_1 measured to coincide with these periods.

Determination of normal ranges for both glycosylated serum protein and HbA_1 were calculated with the control subjects' data. For the purpose of analysis, before the study, 100 mg/dl was selected as the cutoff value for the upper limits of normal for overall mean blood glucose values for any 2-week period. Univariate and bivariate statistical analysis was performed. For categoric data χ^2 and Fisher's exact tests were used and for continuous data the Student t test was used. The association between HbA_1 and glycosylated serum protein and glucose data was measured by Pearson's correlation coefficient.

Results

Twenty-six women with gestational diabetes and 18 nondiabetic women participated in the study. A comparison of the two groups showed no significant difference for maternal age, gestational age at delivery, parity, and maternal weight. A significant difference was found in the incidence of obesity at entry into the study, with the group with gestational diabetes having almost a threefold higher rate (Table I). Evaluation of self-monitored blood glucose testing for the group with gestational diabetes revealed an average of 6.5 ± 1 per day.

In period 1 the association between HbA_1, glycosylated serum protein, and glucose level was measured. As shown in Table II, there was no relationship when the mean fasting blood glucose level (by self-monitored

Table II. The correlation between HbA_1, GSP, and blood glucose level in period 1

	GSP (n = 26)			*HbA_1 (n = 26)*		
Blood glucose	*r*	*r^2*	*p Value*	*r*	*r^2*	*p Value*
Fasting	0.15	0.02	NS	0.19	0.04	NS
Preprandial	0.42	0.18	<0.05	0.56	0.31	<0.01
2-Hr postprandial	0.54	0.29	<0.05	0.66	0.44	<0.01

GSP, glycosylated serum protein.

Table III. The correlation between HbA_1, GSP, and blood glucose level in period 5

	GSP (n = 18)			*HbA_1 (n = 23)*		
Blood glucose	*r*	*r^2*	*p Value*	*r*	*r^2*	*p Value*
Fasting	0.69	0.48	0.05	0.52	0.27	0.001
Preprandial	0.67	0.45	0.05	0.58	0.34	0.001
2-Hr postprandial	0.47	0.22	0.05	0.51	0.26	0.001
Grand mean	0.52	0.27	0.05	0.51	0.26	0.001

GSP, glycosylated serum protein.

blood glucose) was associated with either glycosylated serum protein or HbA_1. A significant correlation was uncovered for the relationship between preprandial blood glucose values and glycosylated serum protein, as well as HbA_1. The 2-hour postprandial values were also significantly correlated with the glycosylated serum protein and HbA_1 for the period.

We repeated the analysis of the relationship between HbA_1 and glycosylated serum protein and verified blood glucose data for periods 2 through 5. The level of metabolic control throughout pregnancy as gauged by self-monitored blood glucose values for each study subject with gestational diabetes remained stable, resulting in an overall mean of 92 ± 28 mg/dl. A significant positive correlation was found between HbA_1, fasting, preprandial, postprandial, and overall mean values. In Table III we summarize the data for period 5 (which was virtually the same as that for the previous periods). During this period, capillary blood glucose values and glycosylated serum protein values were determined for 18 subjects with an overall correlation of $r = 0.52$ ($p < 0.05$). For the same period a correlation coefficient of $r = 0.51$ ($p < 0.001$) was calculated for HbA_1. The difference in level of significance was the result of the unequal number of study subjects.

Fig. 1 shows that for all subjects both the mean glycosylated serum protein and the mean HbA_1 levels remained stable throughout the study period. Greater variability at each period was found for the glycosylated serum protein values compared with the parallel HbA_1 values.

We next examined the relationship between HbA_1 and capillary blood glucose values for cumulative periods of 4, 6, and 8 weeks, respectively. We found a similar level of predictability for all time intervals as that calculated for 2 weeks ($r = 0.51$ to 0.62). Examination of glycosylated serum protein in relationship to glucose level at any 1- or 2-week consecutive interval after period 1 demonstrated a significant correlation at all points of glucose determination during the day ($r = 0.47$ to 0.69).

Finally, the distribution of HbA_1 and glycosylated serum protein in relation to mean blood glucose levels and metabolic control was considered. Illustrated in Fig. 2 is the scattergram of the mean HbA_1 versus mean blood glucose value for each patient for the five periods. Fig. 2 shows that 83 of the 117 points fall within the lower left quadrant bordered by the upper limits of normal HbA_1 and mean blood glucose values (based on the control values). Thus 71% of the time there was consistency between HbA_1 and mean blood glucose values, both simultaneously falling within the normal range. In Fig. 3 the same analysis is used to show that 39 of the 89 points fall within the limits of the normal range for glycosylated serum protein and mean blood glucose values (based on the control values). We therefore found that 43% of the time the mean blood glucose and mean glycosylated serum protein values coincided.

Comment

Traditionally HbA_1 has been considered a predictor of long-term glycemic control.[10] The rationale for this assumption is that HbA_1 is dependent on the life span of the red blood cell, which averages 120 days.[5,6] However, it has been reported that the representative time span for the predictability of HbA_1 ranges from 4 to

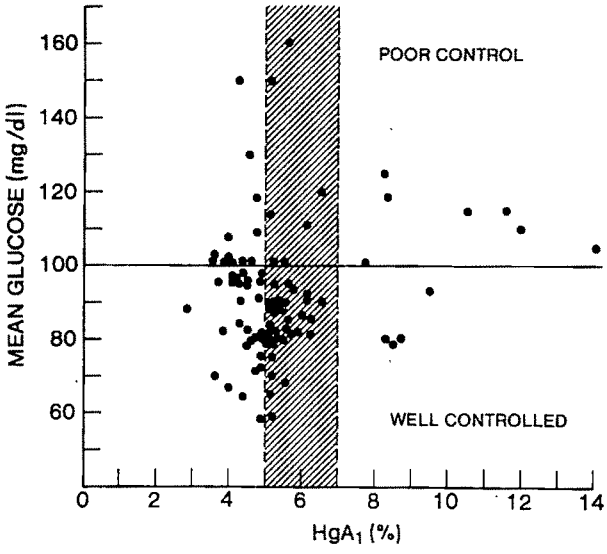


Fig. 2. Distribution of HbA_1 results in relationship to mean blood glucose levels.

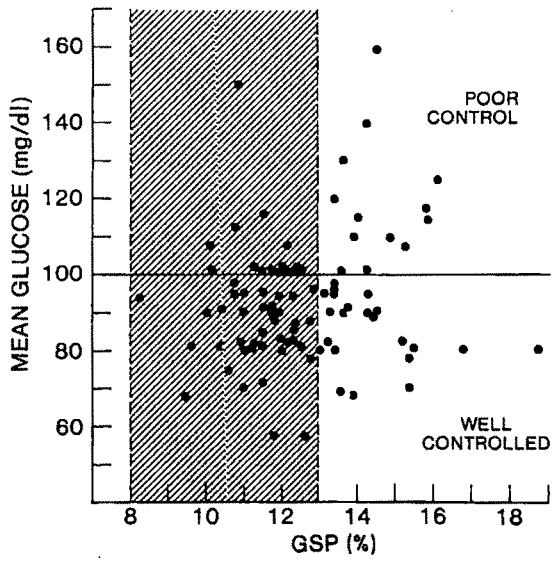


Fig. 3. Distribution of glycosylated serum protein *(GSP)* results in relationship to mean blood glucose levels.

12 weeks.[11-13] To date, the question of what is the best representative time interval for HbA_1 as a predictor of long-term control has not been fully answered. Our study attempted to answer this question by examining the relationship between HbA_1 and glycemic control at 2-, 4-, 6-, and 8-week intervals throughout pregnancy complicated by gestational diabetes. We found that the predictive value of HbA_1 did not improve when the length of time covered with the assay was extended by 2, 4, and 6 weeks. Thus the use of HbA_1 as a long-term predictor must be questioned.

Glycosylated serum protein is traditionally recognized as an evaluator of short-term glycemic control.[3, 4] This has not been studied extensively during pregnancy. Only two studies have examined the role of glycosylated serum protein in gestational diabetes. The first addressed the role of glycosylated serum protein as a diagnostic tool for gestational diabetes.[14] In this study, glycosylated serum protein was found to have a poor correlation to the oral glucose tolerance test. The second study investigated the association between glycosylated serum protein and metabolic control. However, the duration of time, level of control, and method used to obtain glucose data were not specified.[15] The results of the latter study revealed that glycosylated serum protein values were not consistently elevated in patients with gestational diabetes. In our study a predictability of approximately 25% was found for all intervals throughout the study period. Furthermore, there was a high percentage of false positive and false negative test results in relationship to mean glucose values (as was found in HbA_1). Thus a question must be raised as to the clinical usefulness of glycosylated serum protein for verification of metabolic control.

It is our contention that with the use of a memory-based reflectance meter to assure reliable glucose data, we were able to study the true relationships in gestational diabetes of HbA_1 and glycosylated serum protein to metabolic control in a longitudinal manner. This led us to conclude that despite a statistically positive correlation between HbA_1 and mean blood glucose levels, and between glycosylated serum protein and mean blood glucose levels, their use as a clinical tool is limited because of poor predictability.

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Early third-trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term

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The purpose of this study was to determine whether an early third-trimester fetal abdominal circumference measurement can be used in patients with gestational diabetes to predict the presence or absence of macrosomia and labor dystocia at term. The predictive accuracy of a 30- to 33-week abdominal circumference measurement was tested, using the ninetieth percentile as the discriminant point. The study consisted of 201 patients with gestational diabetes who maintained weekly fasting glucose levels <100 mg/dl and 2-hour postprandial glucose levels <120 mg/dl with dietary management alone. The predictive accuracy of a 30- to 33-week fetal abdominal circumference measurement was 96.4% for ruling out macrosomia and 56.3% for predicting macrosomia. Patients with fetal abdominal circumference measurements >the ninetieth percentile at 30 to 33 weeks had a significantly increased incidence of cesarean section for failure to progress, shoulder dystocia, and birth trauma, whereas patients with abdominal circumference measurements ≤the ninetieth percentile were at no greater risk than the general population. These results suggest that patients with non-insulin-dependent gestational diabetes with fetal abdominal circumference measurements ≤the ninetieth percentile at 30 to 33 weeks are not at increased risk for macrosomia, cesarean section, or birth trauma at term, as long as their weekly glucose testing remains within normal limits. Efforts to decrease the incidence of macrosomia and its attendant risks should focus on those gestational diabetic patients whose fetal abdominal circumference >the ninetieth percentile at 30 to 33 weeks. (Am J Obstet Gynecol 1987;157:703-8.)

Key words: Gestational diabetes, macrosomia, ultrasound screening

Gestational diabetes is associated with a significantly increased incidence of fetal macrosomia[1-3] and its attendant risks, including traumatic delivery, shoulder dystocia, and cesarean section. In an attempt to decrease the incidence of gestational diabetes–related morbidity, recent studies have investigated the use of either prophylactic insulin[4] or intensive maternal home glucose monitoring[5] for all patients with gestational diabetes. These studies have reported a significantly decreased incidence of macrosomia and labor morbidity. However, it is not certain whether the logistic constraints of having all patients with gestational diabetes on these ancillary therapies does not outweigh the benefits, particularly because the majority of gestational diabetics treated with dietary control alone and weekly glucose surveillance do not have infants with macrosomia.

Given the potential benefits, as well as the considerable logistic constraints, it would be extremely ben-

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eficial if we could identify those patients who might benefit from ancillary treatment and those for whom it is unnecessary. Therefore the purpose of this study was to determine whether early third-trimester ultrasonographic assessment of the fetal abdominal size can be used as a screening tool to identify which patients with gestational diabetes may potentially benefit from additional therapies.

Material and methods

The study group consisted of 201 patients with gestational diabetes followed at the Cedars-Sinai antepartum testing unit between January 1984 and October 1986. Gestational diabetes was diagnosed according to the criteria of O'Sullivan and Mahan,[6] modified to correct for the methodologic change from the Somogyi-Nelson method to glucose oxidase and for measurement of plasma rather than whole blood glucose.[7] With a 100 gm glucose tolerance test, gestational diabetes was diagnosed if two or more of the following criteria were met or exceeded: fasting, 95 mg/dl; 1 hour, 180 mg/dl; 2 hours, 155 mg/dl; and 3 hours, 140 mg/dl.

Patients were managed with a diabetic pregnancy diet (30 to 35 kcal/kg of ideal body weight and consisting of 25% fat, 25% protein, and 50% complex carbohydrate) and weekly fasting and 2-hour postprandial plasma glucose measurements. To be included in the study, gestational diabetes had to be diagnosed before 34 weeks' gestational age, and the weekly fasting and 2-hour postprandial plasma glucose values had to be maintained at <100 mg/dl and 120 mg/dl, respectively, on diabetic diet alone. In addition, patients had to have accurate early pregnancy dating criteria (accurate date of last menstrual period and ultrasonographic examination performed in the first or second trimester) and they had to have delivered beyond 36 completed weeks of gestational age.

All study patients underwent ultrasonographic fetal measurements between 30 and 33 completed weeks of gestational age, with an ATL linear-array real-time ultrasound scanner, model 4000 (Advanced Technology Laboratories, Seattle, Washington). The techniques used for abdominal circumference measurement and calculation were the same as those reported by Shields et al.[8] The accuracy of a 30- to 33-week fetal abdominal circumference measurement for predicting the presence or absence of fetal macrosomia, labor dystocia, shoulder dystocia, or birth trauma at term was tested using the nintieth percentile as the discriminant point.

Abdominal circumference percentile ranks were assigned according to the previously established reference growth curves for the Cedars-Sinai population. The general formula used for the predicted abdominal circumference at each gestational age was: abdominal circumference = $-12.67 + 1.59$ (gestational age) $- 0.0102$ (gestational age)^2 ($R^2 = 0.945$, SE of the estimate = 1.828). The individual confidence interval for the ninetieth percentile was calculated according to the following formula: ninetieth percentile = predicted abdominal circumference + 1.282 × square root (1 + 1/347 + [gestational age − 29.08]^2/18723) × 1.889. The abdominal circumference values representing the ninetieth percentile were 28.3, 29.3, 30.2, and 31.1 cm, respectively, for 30, 31, 32, and 33 weeks' gestation.

Macrosomia was considered to be a birth weight >the ninetieth percentile for the Cedars-Sinai patient population. The standard reference curves for our population were developed by a regression analysis of the birth weights from 10,135 singleton pregnancies that included all deliveries of normal live-born neonates for the years 1983 and 1984. The regression analysis was stratified and the best fit achieved by use of an inflection point of 35.0 weeks, selected to give the least SE of the estimate.

The labor and delivery and neonatal records were reviewed for each patient in the study. Delivery outcomes were compared between patients with fetal abdominal circumferences ≤the ninetieth percentile at 30 to 33 weeks' gestational age and those with abdominal circumferences >the ninetieth percentile. Labor dystocia was defined as failure to progress in labor for >2 hours, despite adequate contractions, once the active phase of labor had begun (cervical dilatation >3 to 4 cm). Shoulder dystocia was considered to be present if fetal shoulder impaction at delivery required additional maneuvers other than head traction. Birth trauma included fetal fractures, hematomas, and soft tissue injuries.

Statistical analyses were performed by χ^2, Fisher's exact test, or the unpaired t test, as appropriate. A p value <0.05 was considered significant.

Results

In the overall study population of 201 patients, infants with a birth weight >the ninetieth percentile had significantly increased incidences ($p < 0.001$) of cesarean section for failure to progress in labor (41.7% versus 14.3%), shoulder dystocia (16.7% versus 0.7%), and birth trauma (3.6% versus 20.8%), compared with infants with a birth weight ≤the ninetieth percentile. Of the 31 infants with a birth weight >4000 gm, 20 received a trial of labor, and this resulted in eight cesarean sections for failure to progress, four cases of shoulder dystocia, and five infants with birth trauma.

When the study patients were grouped according to fetal abdominal circumference measurements at 30 to 33 weeks' gestational age, there were no significant differences between the groups in terms of the mean maternal age, percentage of nulliparous women, mean prepregnancy weight, mean gestational age that dia-

Table I. Maternal statistical data

	AC ≤ ninetieth percentile (n = 137)	*AC > ninetieth percentile (n = 64)*
Maternal age (yr ± SD)	31.2 ± 5.4	30.9 ± 5.2
% Nulliparous	39	41
Prepregnancy weight (pounds)	126 ± 23	123 ± 25
Mean (±SEM) gestational age at diagnosis (yr)	30.45 ± 1.06	30.42 ± 1.07
Mean (±SEM) gestational age at screening ultrasound (wk)	31.56 ± 1.04	31.87 ± 1.10
Mean (±SEM) gestational age at delivery (wk)	39.52 ± 1.60	38.7 ± 1.89

AC, abdominal circumference.

Table II. Neonatal outcome of the study groups

	AC ≤ ninetieth percentile (n = 137)	*AC > ninetieth percentile (n = 64)*
Mean (±SEM) birth weight (gm)*	3373 ± 342	3851 ± 549
No. >ninetieth percentile (%)*	5 (3.6)	36 (56.3)
No. >4000 gm (%)*	5 (3.6)	26 (40.6)

AC = abdominal circumference.
*$p < 0.001$.

Table III. The predictive values, sensitivity, and specificity of fetal AC measurements at 30 to 33 weeks for predicting macrosomia at term

AC measurements	*Macrosomia*	*Normal BTWT*	*PV+*	*PV−*	*Sens*	*Spec*
AC > ninetieth percentile	36	28	56.3	96.4	87.8	82.5
AC ≤ ninetieth percentile	5	132				

AC, abdominal circumference; BTWT, birth weight; PV+, predictive value of AC > ninetieth percentile for predicting macrosomia; PV−, predictive value of AC ≤ ninetieth percentile for predicting the absence of macrosomia; Sens, sensitivity; Spec, specificity.

betes was diagnosed, or the timing of the screening fetal abdominal circumference measurement (Table I). However, at term the group that had a fetal abdominal circumference >the ninetieth percentile between 30 and 33 weeks had a significantly higher ($p < 0.001$) mean birth weight, percentage of infants with birth weight >the ninetieth percentile, and percentage of infants weighing >4000 gm (Table II).

The accuracy of a 30- to 33-week fetal abdominal circumference for predicting the presence or absence of macrosomia at term (using the ninetieth percentile as the discriminant point) is presented in Table III. The positive predictive value of an abdominal circumference >the ninetieth percentile for predicting a fetal birth weight >the ninetieth percentile was only 56.3%, and for predicting a birth weight >4000 gm it was only 40.6%. Conversely, the negative predictive value of an abdominal circumference ≤the ninetieth percentile for predicting the absence of macrosomia was 96.4%. The sensitivity of a fetal abdominal circumference >the ninetieth percentile for identifying those patients with eventual macrosomia was 87.8%, whereas the specificity of a fetal abdominal circumference ≤the ninetieth percentile for identifying those patients without macrosomia was 82.5%.

Only one elective cesarean was performed for presumed fetal macrosomia. Of the 164 patients who underwent a trial of labor, the group with fetal abdominal circumferences >the ninetieth percentile between 30 and 33 weeks did not have an overall significantly increased incidence of cesarean section for failure to progress, compared with patients with abdominal circumferences ≤the ninetieth percentile (Fig. 1). However, patients with fetal abdominal circumferences >the ninetieth percentile did have a significantly increased incidence of cesarean section for failure to progress ($p < 0.001$) beyond 39 completed weeks' gestational age. Overall, they also had significantly increased incidences of shoulder dystocia and birth trauma ($p < 0.001$) compared with patients with fetal abdominal circumferences ≤the ninetieth percentile (Fig. 2). When compared with the general Cedars-Sinai

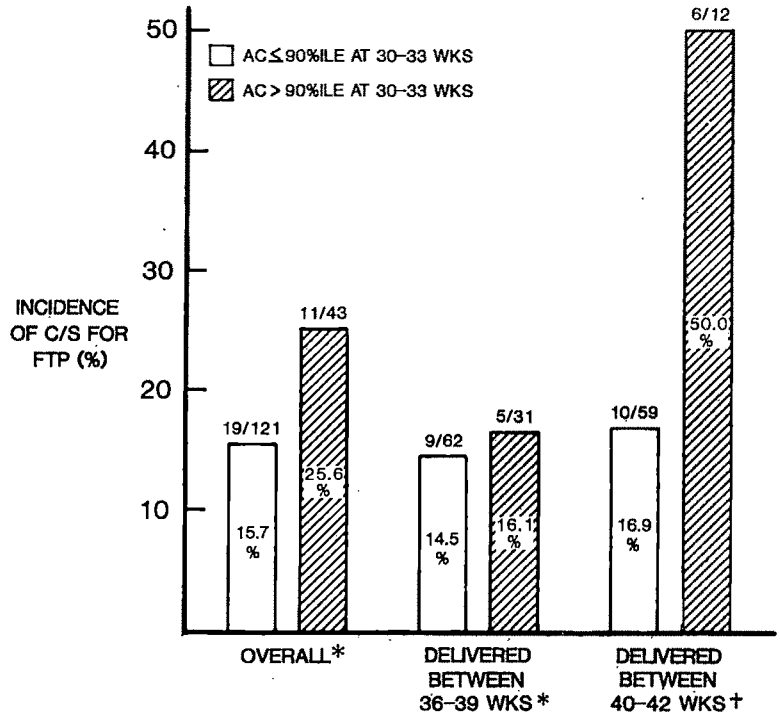


Fig. 1. The incidence of cesarean section for failure to progress in patients given a trial of labor. *not significant; †$p < 0.001$; *AC*, abdominal circumference; *C/S*, cesarean section; *FTP*, failure to progress.

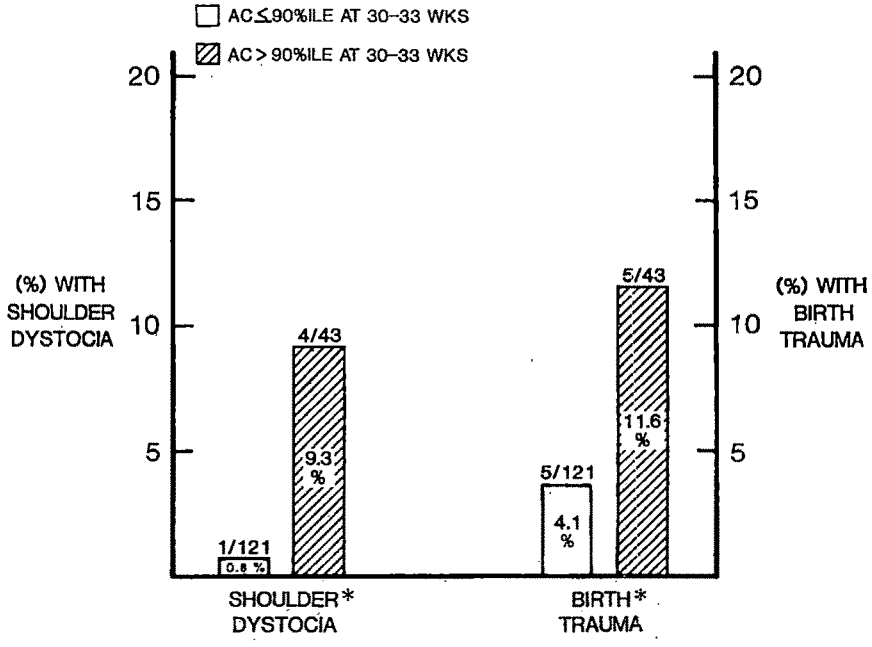


Fig. 2. The incidence of shoulder dystocia and birth trauma in patients given a trial of labor. *$p < 0.001$; *AC*, abdominal circumference.

term population, the group with fetal abdominal circumferences ≤the ninetieth percentile was not at increased risk for cesarean section as a result of failure to progress (15.7% versus 16.6%) or shoulder dystocia (0.8% vs 0.8%).

Comment

Gestational diabetes has been shown to result in a significantly increased incidence of fetal macrosomia and its associated risks, including labor dystocia and trauma.[1-5] Although recent investigators have shown a

decrease in macrosomia-related morbidity, by either treating all gestational diabetics with prophylactic insulin[4] or increasing glucose surveillance,[5] the majority of gestational diabetics do not suffer from macrosomia-related morbidity when managed simply with dietary control and weekly glucose testing alone. This study, therefore, was undertaken in an attempt to determine whether early third-trimester screening can identify which patients may potentially benefit from ancillary therapy.

Previous investigators have attempted, at term, to use ultrasonographic parameters of fetal size to identify patients with fetal macrosomia,[9-11] as well as those at risk for shoulder dystocia.[12] Others have studied diabetic patients during the preterm period in an attempt to predict which ones are destined to become macrosomic at term.[13, 14] We chose a gestational age of 30 to 33 weeks as our screening time period because a study by Ogata et al.[13] showed that in diabetic patients with eventual macrosomia at term, the fetal abdomen initially began to demonstrate accelerated growth between 28 and 32 weeks' gestational age. In addition, this is a time period that is considered to be early enough in pregnancy for insulin therapy to be beneficial, if needed.[4]

The fetal abdominal circumference was chosen as the screening parameter because it has been shown to be the single most accurate fetal growth parameter for predicting macrosomia in patients with accurate dating.[11, 13] This is likely because the fetal abdomen is the body part most affected by diabetes-induced accelerated growth.[13] The ninetieth percentile was chosen because it has been shown to be the best abdominal circumference percentile value to use for predicting macrosomia.[11, 13]

Our data agreed with these findings. The fetal abdominal circumference in this study had better predictive values for the presence or absence of macrosomia at term compared with the other individual fetal structural parameters measured including biparietal diameter, head circumference, and femur length, or the calculated estimated fetal weight,[15] head circumference/abdominal circumference ratio, or femur length/abdominal circumference ratio. However, the predictive value for fetal macrosomia was not statistically improved by combining an estimated fetal weight >the ninetieth percentile with an abdominal circumference >the ninetieth percentile, in contrast to the findings of Tamura et al.[17] This may be because their values were obtained closer to term, at which time the progressive accelerated fetal growth may have resulted in both the fetal abdominal circumference and biparietal measurements reaching higher percentile ranks.

The abdominal circumference ninetieth percentiles calculated for our population between 30 and 33 weeks are virtually the same as those of Deter et al.[16] In addition, the birth weight ninetieth percentiles at term were within 20 to 30 gm of the overall California birth weight curves.[17] Therefore these tables can be used interchangeably with ours for the purposes of this study. However, we agree in principle with Deter et al. that to optimally evaluate specific populations, individual growth and birth weight curves should be determined.

The study results suggest that an ultrasonic fetal abdominal circumference measurement ≤the ninetieth percentile between 30 and 33 weeks' gestation is a highly accurate predictor for the absence of either fetal macrosomia (Table III), dystocia, or birth trauma (Figs. 1 and 2) at term. It appears that if the influence of gestational diabetes has not resulted in accelerated fetal growth by 30 to 33 weeks, it is unlikely that patients with normal weekly glucose testing will develop macrosomia and they can be treated with dietary management alone.

Conversely, a fetal abdominal circumference >the ninetieth percentile between 30 and 33 weeks' gestation was not an accurate predictor of macrosomia at term (Table III). Therefore we cannot reliably predict the delivery outcome in such patients. However, an abdominal circumference >the ninetieth percentile did have a high sensitivity for identifying patients with eventual macrosomia (Table III), and it was associated with a significantly increased incidence of labor dystocia, shoulder dystocia, and birth trauma (Figs. 1 and 2).

If ancillary therapies are to be employed, patients with a fetal abdominal circumference >the ninetieth percentile at 30 to 33 weeks appear to be the ones who may benefit. These patients may also benefit from induction of labor before 40 weeks' gestational age, because the risk for failure to progress in labor did not statistically increase until that point. Focusing our efforts on those patients with gestational diabetes with a fetal abdominal circumference >the ninetieth percentile at 30 to 33 weeks may enable us to decrease the incidence of gestational diabetes–related macrosomia and morbidity while minimizing the inconvenience to the overall population with gestational diabetes and the logistic constraints to the medical care system.

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Chorionic villus sampling is associated with normal fetal growth

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Seventy-eight singleton term neonates, delivered of women who were 35 years of age or older after chorionic villus sampling at 9 to 11 weeks' gestation, were compared with 1335 singleton control neonates delivered at term of mothers 35 years of age or older before the clinical application of chorionic villus sampling at our institution. No statistically significant differences were found in birth weight, crown-heel length, or ponderal index between control neonates and neonates delivered after chorionic villus sampling. The sample sizes necessary to detect a 5% difference in population means at an alpha error level of 0.05 and beta error level of 0.1 were calculated for the measured variables and achieved before analysis. This study suggests that patients undergoing chorionic villus sampling are not at increased risk for the development of decreased fetal growth or intrauterine growth retardation. (AM J OBSTET GYNECOL 1987;157:708-12.)

Key words: Chorionic villus sampling, intrauterine growth retardation, fetal growth

Decreased fetal growth and intrauterine growth retardation are theoretical risks of chorionic villus sampling. The U.S. Food and Drug Administration has required patients undergoing chorionic villus sampling to be counseled of this risk. Early reports suggested that the percentage of infants delivered after chorionic villus sampling with birth weights below the tenth percentile is no different from that in the general population.[1,2] However, comparisons of weight, length, and proportionality between neonates delivered after first-trimester chorionic villus sampling and a suitable control population have not been reported. The purpose of this study was to investigate the effect of chorionic villus sampling on fetal growth as assessed by the neonatal evaluation of birth weight, crown-heel length, and ponderal index in the term pregnancy to determine whether a decrease in size or distribution of these obstetric outcome variables could be shown.

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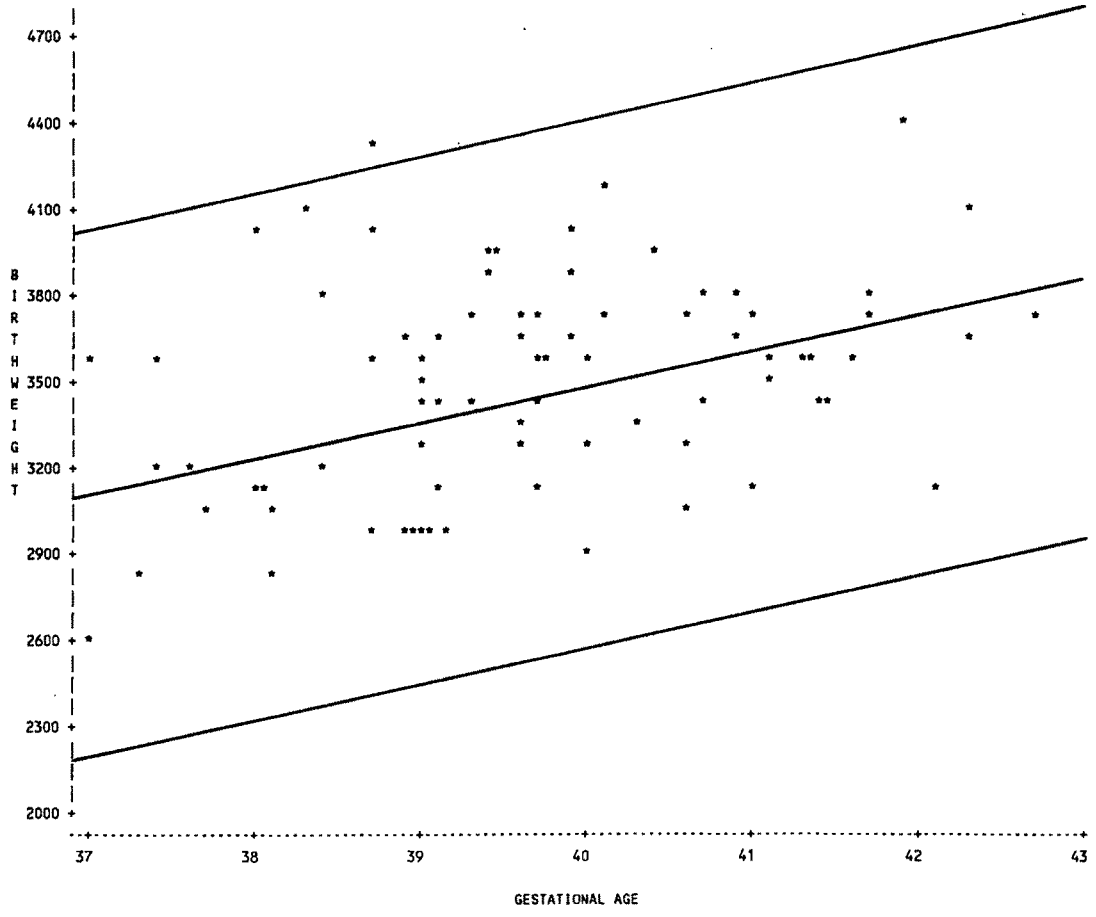


Fig. 1. The distribution of neonatal birth weight (measured in grams) versus gestational age from pregnancies having had chorionic villi sampling at 9 to 11 weeks' gestation. Predicted mean, ninety-fifth and fifth percentiles for the control population are superimposed on the graph.

Table I. Variables studied in the control and chorionic villi sampling populations

	Controls	*Chorionic villi sampling*	*p Value*
No.	1335	78	—
Gestational age (wk) (mean ± SD)	39.8 ± 1.3	39.7 ± 1.4	0.33*
Maternal age	36.9 ± 1.93	36.9 ± 2.59	0.78*
Parity	1.18 ± 1.39	1.14 ± 1.02	0.78*
Birth weight (gm)	3458 ± 494	3503 ± 380	0.31*
Crown-heel length (cm)	51.8 ± 2.6	51.7 ± 2.0	0.49*
Ponderal index	2.48 ± 0.29	2.54 ± 0.27	0.07*

*Not statistically significant.

Material and methods

Seventy-eight term (36 to 42 completed gestational weeks) neonates delivered of patients who had undergone transcervical chorionic villus sampling at 9 to 11 weeks' menstrual age were evaluated at delivery for birth weight and crown-heel length. The ponderal index (birth weight/[crown-heel length]^3 · 100) was calculated. All infants were products of singleton pregnancies and had normal karyotypes. The maternal age at delivery was between 35 and 45 years. Aspiration of chorionic villi was performed with a 16-gauge catheter (Becton-Dickinson) with a No. 7 stainless steel obturator (Ethicon). Chorionic villi sample sizes ranged from 3 to 75 mg with a mean of 21.4 ± 11.1 mg (mean ± SD). The maximum number of catheter insertions per patients was three (mean 1.6 ± 0.7).

A control population of 1335 phenotypically normal neonates from singleton pregnancies delivered at term

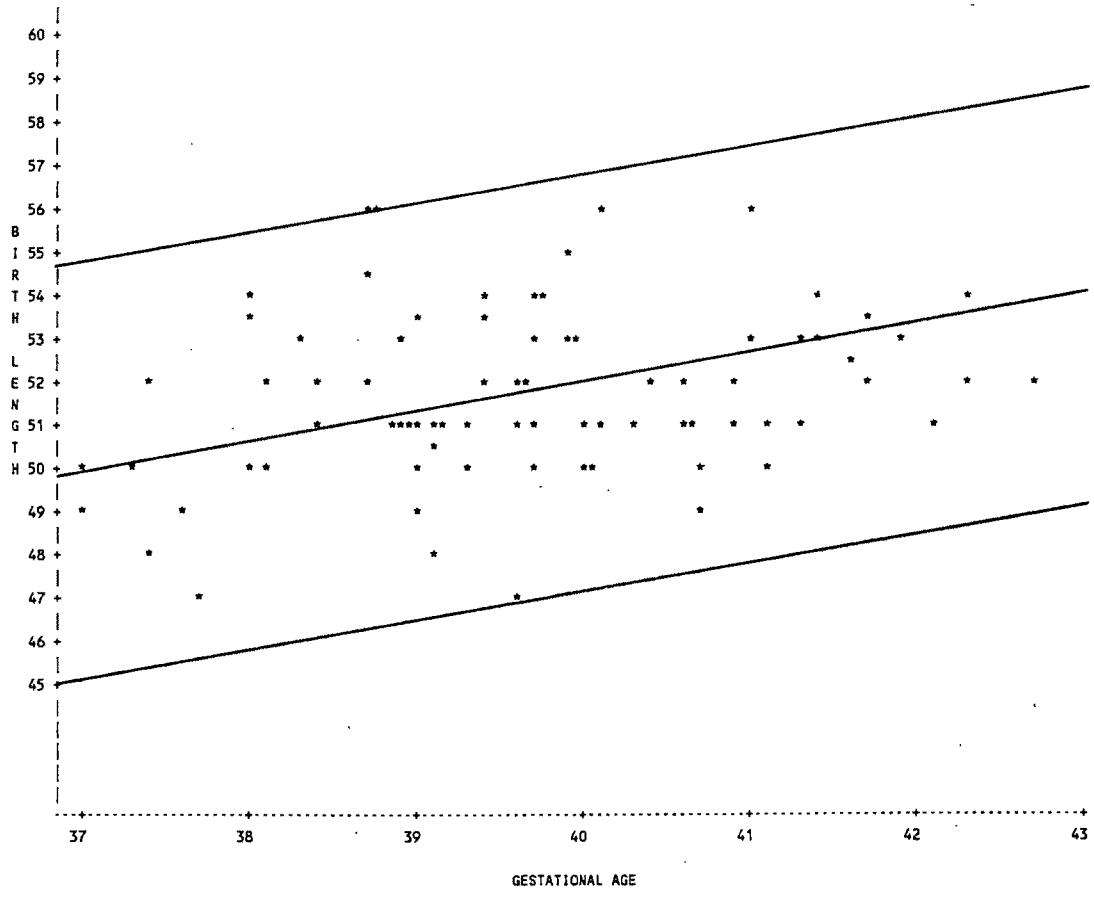


Fig. 2. The distribution of crown-heel lengths (measured in centimeters) versus gestational age of neonates from pregnancies having had chorionic villi sampling at 9 to 11 weeks' gestation. Predicted mean, ninety-fifth and fifth percentiles for the control population are superimposed on the graph.

of mothers between 35 and 45 years of age was evaluated in a similar manner to the study population. These control data were developed from a population of patients, similar to the patients having chorionic villus sampling, delivered at Cedars-Sinai Medical Center during the 2-year period immediately preceding the clinical application of chorionic villus sampling at this center. The means and standard deviations were calculated for maternal age, gestational age, birth weight, crown-heel length, and ponderal index in each population and compared by means of Student's *t* test.

In addition, the predicted mean, ninety-fifth, and fifth percentiles for the control population were determined and the distribution of each variable in the study population was plotted against gestational age. With an alpha error level of 0.05 and beta error level of 0.1, the sample size necessary to detect a 5% difference in population means was calculated for the measured variables and achieved before analysis.

Results

The differences between all variables evaluated for both study and control populations at delivery were not statistically significant. These values and their respective p values are summarized in Table I. The birth weights, crown-heel lengths, and ponderal indices were normally distributed for both chorionic villus sampling and control populations. The distributions of birth weight, crown-heel length, and ponderal index versus gestational age are plotted for the chorionic villus sampling patient population in Figs. 1, 2, and 3. The predicted mean, ninety-fifth and fifth percentiles for the control population are superimposed on the appropriate figures.

Comment

Chorionic villus sampling is rapidly emerging as a diagnostic alternative to amniocentesis. The advantage of chorionic villus sampling is that it is done in the first trimester and thereby provides earlier diagnosis. In cases in which the fetus is affected, earlier diagnosis allows for safer pregnancy termination. Initial concerns about chorionic villus sampling included a theoretic risk for placental complications (abruptio placentae or placenta previa) and intrauterine growth retardation attributable to trauma from insertion of the catheter

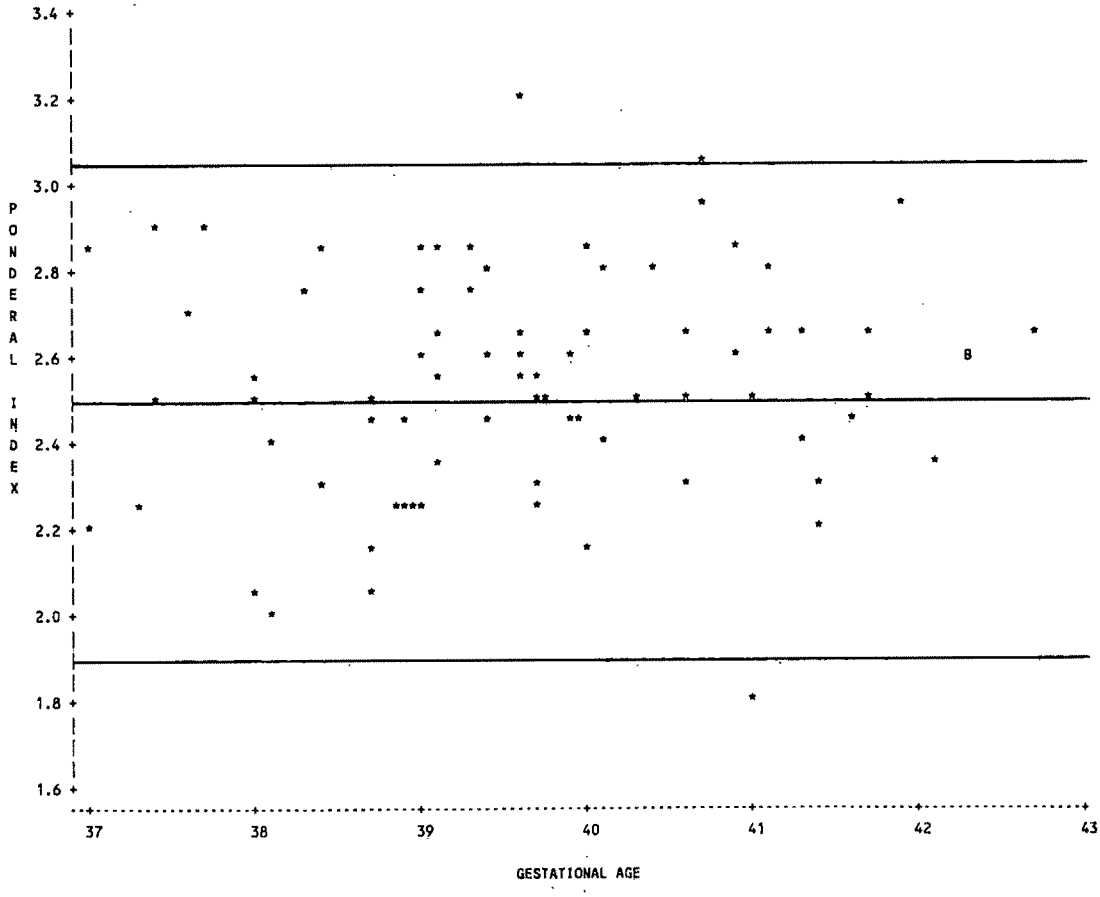


Fig. 3. The distribution of ponderal index versus gestational age of neonates from pregnancies having had chorionic villi sampling at 9 to 11 weeks' gestation. Predicted mean, ninety-fifth and fifth percentiles for the control population are superimposed on the graph.

and/or aspiration of sufficient chorionic villi to disrupt placental growth or function.[3] In a clinical evaluation of 300 births after chorionic villus sampling in Milan, Brambati et al.[2] reported that the frequencies of placental complications and low birth weight were similar to those found in the general Italian population. However, no statistical comparison between the mean birth weights in the chorionic villus sampling group and general population was presented.

Because many biologic factors including maternal age and parity as well as environmental factors are known to influence fetal growth,[4] the general population is not a suitable control group for the evaluation of the growth in neonates who have undergone chorionic villus sampling. The association of increased maternal age with parity and the high frequency of maternal age as an indication for chorionic villus sampling are factors that should be considered when evaluating growth after chorionic villus sampling, particularly when the most frequent indication for chorionic villus sampling at our institution (86%) and others[5, 6] is advanced maternal age (35 years or older at delivery). For this reason, both the chorionic villus sampling and control populations were restricted to patients aged 35 years or older at the time of delivery in our study. There was no significant increase in either maternal age or parity between control and chorionic villus sampling populations (Table I). The chorionic villus sampling and control groups in our study also were similar with respect to the gestational age at delivery. The percentage of patients undergoing amniocentesis in the control population is not known. However, since amniocentesis has not been shown to alter the outcome variables we were studying,[7] we believe that our control population is not biased by the absence of this information.

Our study demonstrates that there are no significant differences in birth weight, length, or proportionality at term between normal control infants and infants who undergo chorionic villus sampling at 9 to 11 weeks' menstrual age. It is noticeable that the p value for the ponderal index, which is a measurement of disproportionality in the neonate, is 0.07. However, the increase in this value is not a possible sign of growth retardation but reflects the opposite finding, resulting from a small increase in neonatal weight and a decrease in the neonatal length. The distribution of the variables of fetal

growth evaluated in this study are evenly distributed about the mean and no increase in values above or below the ninety-fifth and fifth percentiles for each variables are present (Figs. 1-3).

We calculated the sample size needed to detect a 5% difference in each of the outcome variables for an alpha error level of 0.05 and beta error level of 0.1. These sample sizes were exceeded for all variables studied. Therefore we conclude that patients undergoing first-trimester chorionic villus sampling are not at increased risk for the development of decreased fetal growth nor at increased risk for intrauterine growth retardation.

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Umbilical blood waveforms in fetal surveillance of twins

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Umbilical arterial velocity waveforms were recorded in 69 pairs of twins by means of continuous-wave Doppler analysis and an Angioscan spectrum analyzer. A systolic/diastolic ratio was measured for each twin. The sum of the systolic/diastolic ratio was calculated and the difference between each twin was expressed as the delta systolic/diastolic ratio. Significant differences in delta systolic/diastolic and neonatal weight were previously set at 0.4 and 350 gm, respectively. Sonography was used for fetal age confirmation and diagnosis of fetal growth retardation. All neonates were weighed; at birth gestational age was assigned by Dubowitz criteria and categorized as adequate-, small-, or large-for-gestational age. The sensitivity, specificity, and predictive value of the delta systolic/diastolic ratio were determined. The perinatal implications of this test in the management of twin pregnancy are discussed. (Am J Obstet Gynecol 1987;157:712-5.)

Key words: umbilical artery waveforms, blood flow, twins

Twin pregnancy is characterized by high perinatal mortality and morbidity. A major causative factor for these difficulties is intrauterine growth retardation in one member of the twin dyad. Farmakides et al.[1] have shown that umbilical blood waveforms can be used to select the growth-retarded twin. The purpose of this study is to further elucidate the relationship of umbilical blood waveforms to fetal growth and well-being in twin dyads. An attempt was made to correlate differences in umbilical blood waveforms between members of the twin dyad to perinatal mortality and serious anomalies.

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Material and methods

A total of 79 pairs of twins were detected by ultrasound before 26 weeks' gestation. Nine patients were excluded because of fetal death before 28 weeks (four) or noncompliance with the protocol (five). Sonographic examination helped to establish or confirm the gestational age. At birth, all babies were weighed and gestational age was assessed by a modification of the criteria developed by Dubowitz et al.[2] Twin dyads were then classified as AGA/AGA, AGA/SGA, SGA/SGA, or AGA/LGA according to the criteria of Naeye et al.[3] for multiple pregnancies. Clinicians referring patients were unaware of the results of umbilical velocimetry.

Table I. Predictability of Δsystolic/diastolic ratio for Δbirth weight

	AGA/AGA (N = 50)	*AGA/SGA (N = 10)*	*SGA/SGA (N = 4)*	*AGA/LGA (N = 4)*
S/D < 0.4				
Wt > 350 gm	39	1	0	0
Wt ≤ 350 gm	1*	0	0	3*
S/D ≥ 0.4				
Wt > 350 gm	6†	0	4†	0
Wt ≤ 350 gm	4	9	0	1

S/D = Systolic/diastolic ratio; Wt = birth weight.
*False negative. Predictive value of Δsystolic/diastolic ratio < 0.4 = 91%.
†False positive. Predictive value of Δsystolic/diastolic ratio = 0.4 = 42%.

All mothers had umbilical blood waveform determinations by means of a 4 MHz continuous-wave Doppler transducer (Angioscan) beginning at 20 to 24 weeks. Umbilical cords were identified by differing rates and by their location with the use of ultrasound just before the application of the Doppler transducer. The systolic/diastolic ratio described by Trudinger et al.[4] was determined for each twin at each visit. Patients were seen biweekly until the 28th week when systolic/diastolic ratios were determined weekly until delivery. Individual absolute systolic/diastolic ratios were recorded for each member of the twin dyad. The difference between the systolic/diastolic values for each twin was determined. The sum of the differences was averaged and called the delta (Δ) systolic/diastolic ratio. A Δsystolic/diastolic ratio ≥ 0.04 was previously established[1] as being associated with a significant birth weight difference (Δ birth weight) of 350 gm or more in twin dyads. We attempted to confirm this relationship. In addition, each twin dyad was scrutinized for the absolute systolic/diastolic values of its members and their possible relation to perinatal death and serious anomalies.

Sonograms for calculation of gestational age were done biweekly during this period of time (28 to 40 weeks). However, if absolute systolic/diastolic ratios were abnormal or previous evidence of divergent growth was present, the sonogram was repeated weekly. We compared the Δsystolic/diastolic ratio with the sonographic evidence for divergent fetal growth. We relied primarily on the birth weight for the analysis of Δsystolic/diastolic ratio sensitivity and predictive value. Ultrasound examination consisted of biparietal diameter, femur length, abdominal circumference, and estimation of fetal weight by the method of Warsof et al.[5] The average number of sonograms was five per dyad.

Results

A total of 69 patients were delivered in the third trimester. Of these, 64 patients were delivered within 5 days of the last blood waveform study. Four patients were delivered 6 to 10 days from the last systolic/diastolic determination and only one was delivered 14 days after the last umbilical blood waveform study. The most frequent type of twin dyad was appropriate for gestational age (AGA/AGA), which was found in 51 of 69 patients (74%). Dyads in which one was AGA and the other small for gestational age (SGA) were found in 10 cases (14%), whereas SGA/SGA and AGA/large for gestational age (LGA) dyads had a 6% frequency each. Frequency distributions and predictive values are shown in Table I.

AGA/AGA dyads (N = 51)

ΔSystolic/diastolic ratio < 0.4 and intradyad Δbirth weight < 350 gm. Thirty-nine sets of twins fell in this category. One stillbirth occurred in a twin dyad in which serial systolic/diastolic ratios averaged 3.4 for both fetuses in biweekly tests from 28 to 35 weeks. Absolute systolic/diastolic ratios over 3.0 persisted at 36 weeks but the Δsystolic/diastolic ratio = 0.22 and Δbirth weight = 70 gm. Divergent fetal growth was suggested by serial sonograms. A nonstress test (NST) was nonreactive 2 days before the death of the stillborn twin. Delivery was delayed by an immature lecithin-sphingomyelin (L/S) ratio. The fused, diamniotic placenta showed triploidy and molar degeneration. Another dyad had a stillbirth at 34 weeks. The Δsystolic/diastolic ratio was 0.17 and the Δbirth weight was 110 gm. The stillborn baby had extensive subarachnoid hemorrhage and hemothorax. The surviving twin had thrombocytopenia and disseminated intravascular coagulation. The absolute systolic/diastolic ratios for each twin were all above 3.0.

Review of serial sonograms revealed concordant fetal growth in 24 patients (63%) and discordant fetal growth in 14 dyads (37%). The latter are false-negative values yielded by sonogram examinations. Discordant fetal growth by sonography was present if the biparietal diameter difference was ≥4 mm after 28 weeks or the estimated difference in Δbirth weight ≥350 gm using the method of Warsof et al.[5] In five patients we thought that biparietal diameter (three) or femur length (two) measurements were unreliable or unobtainable because of fetal position.

ΔSystolic/diastolic ratio ≥ 0.4 and Δbirth weight ≥ 350 gm. There were four patients in this subset. These dyads had a relatively high incidence of death and serious anomalies. One dyad had Down's syndrome in both babies (patient had refused genetic amniocentesis). The Δsystolic/diastolic ratio was 0.5 and the Δbirth weight was 377 gm. Sonograms had detected discordant fetal growth starting at 29 weeks. These infants survived the neonatal period. Another dyad had concordant growth detected by sonography but megalocystis was noted at 26 weeks. The patient returned in premature labor at 30 weeks with a Δsystolic/diastolic ratio of 0.8 and Δbirth weight of 361 gm. The smaller twin died of pulmonary hypoplasia. Absolute systolic/diastolic ratios in this baby were 4.1 and 3.7.

The ultrasound measurements showed concordant growth in three of these four dyads. At birth, the average Δbirth weight = 412 gm (range 370 to 540 gm). It was impossible to obtain a biparietal diameter twice in two fetuses because of fetal position or extreme deflexion of fetal head.

ΔSystolic/diastolic ratio ≥ 0.4 and Δbirth weight < 350 gm. There were seven dyads in this group. These represent false-positive results in the expected relationship between Δsystolic/diastolic ratio and Δbirth weight. Sonograms showed divergent growth in four of these seven dyads (57% false positive). All three sonographic parameters (biparietal diameter, femur length, and abdominal circumference) were obtained. The average Δbirth weight was 137 gm (range 60 to 250 gm). Careful review of individual systolic/diastolic ratios showed only two dyads with absolute systolic/diastolic ratios over 3.0 after 28 weeks. One dyad had a twin with absolute systolic/diastolic ratio of 3.0 but Δsystolic/diastolic ratio of 0.5 for the dyad. This twin suffered neonatal death caused by a single ventricle and tricuspid insufficiency. Another dyad had one twin with absolute systolic/diastolic ratio of 3.0 and Δsystolic/diastolic ratio of 0.7 for the dyad. The mother had gestational diabetes with hyperglycemia.

ΔSystolic/diastolic ratio < 0.4 and Δbirth weight ≥ 350 gm. There was only one AGA/AGA dyad with a false-negative value. The Δsystolic/diastolic ratio was 0.14 for the dyad and Δbirth weight was 410 gm. All individual systolic/diastolic values for each twin were less than 3.0 after 28 weeks. Ultrasonography correctly detected divergent fetal growth at 25 weeks and thereafter.

In summary, the AGA/AGA group contains three dyads with severe anomalies. Two of these dyads had neonatal deaths. The other dyad had Down's syndrome. Two other AGA/AGA dyads had a single stillbirth each. In these two cases the Δsystolic/diastolic ratio < 0.4, but the absolute systolic/diastolic ratio consistently exceeded 3.0 after 28 weeks.

AGA/SGA dyads. There were 10 dyads in this group with an average Δbirth weight of 635 gm overall.

ΔSystolic/diastolic ratio ≥ 0.4 and Δbirth weight ≥ 350 gm. There were nine dyads in this subset indicating a true-positive rate of 100% for Δsystolic/diastolic ratio ≥ 0.4. Only one dyad had a smaller twin with absolute systolic/diastolic ratio in the normal range after 28 weeks. Ultrasonography also forecasted divergent growth in seven of these nine dyads (78%). Only in one occasion was a biparietal diameter not obtainable.

ΔSystolic/diastolic ratio < 0.4 and Δbirth weight < 350 gm. One dyad had Δsystolic/diastolic ratio of 0.2 and Δbirth weight of 110 gm. There was only one absolute systolic/diastolic ratio of 3.0 after 28 weeks. A sonogram had forecasted a significant Δbirth weight of 375 gm 2 days before birth.

This group had no mortality and no anomalies were found.

SGA/SGA dyads. There were four dyads in this group. The Δsystolic/diastolic ratio was equal to or greater than 0.4 in all dyads. The average Δbirth weight was 35 gm (range zero to 110 gm). These were all false-positive values for Δsystolic/diastolic ratio in relation to Δbirth weight. Review of absolute systolic/diastolic values revealed that these fetuses had the highest individual systolic/diastolic values in the series (average absolute systolic/diastolic ratio = 4.0; range 3.0 to 5.0). The cesarean section rate was 100% for this subset. Sonograms showed concordant growth in three dyads and divergent growth in one dyad (75% accuracy).

LGA/AGA dyads. There were four dyads in this group and it had the largest Δbirth weight values (range 400 to 630 gm) for the series. There was one dyad with a Δsystolic/diastolic ratio of 0.7 and a Δbirth weight of 630 gm (true positive). All others had false-negative Δsystolic/diastolic ratio values. These fetuses had the lowest absolute systolic/diastolic values.

Predictive value. Table I shows a high predictive value (91%) of Δsystolic/diastolic ratio < 0.4 for confirming concordant fetal growth. Divergent fetal growth was more difficult to detect by Δsystolic/diastolic ratio. There was only 42% predictive value for Δsystolic/diastolic ratio = 0.4.

Comment

Our study indicates that umbilical blood waveforms are a useful adjunct in the management of twin pregnancies. We found the AGA/AGA twin dyad was the most common dyad (74%) in our series. The entire perinatal death and all serious anomalies were also found in this group. This was surprising since we expected both the AGA/SGA and SGA/SGA dyads to have a worse prognosis. In two dyads with individual still-

births, persistent absolute systolic/diastolic ratios over 3.0 suggested placental dysfunction. The Δsystolic/diastolic ratio was not able to differentiate the twin at risk in these two dyads but there was no significant Δbirth weight present. Two neonatal deaths were found among the four AGA/AGA dyads with Δsystolic/diastolic ratio ≥ 0.4 and Δbirth weight ≥ 350 gm. These latter findings agreed with our expectations for the test as a sign of placental dysfunction and an indicator of divergent fetal growth.[6] Ultrasound examinations were the least reliable (59% accuracy) among these AGA/AGA dyads.

The Δsystolic/diastolic ratio correctly detected significant weight differences in 9 of 10 AGA/SGA fetuses and identified the one dyad with Δbirth weight < 350 gm in that group. Serial sonograms had 78% accuracy in this group. Although the seven SGA/SGA dyads showed falsely high Δsystolic/diastolic ratio in all cases, review of the absolute systolic/diastolic values showed that they had persistently high absolute systolic/diastolic ratios.

All four SGA/SGA dyads had falsely high Δsystolic/diastolic ratios with no significant birth weight differences or perinatal morbidity. This was confusing but the number is small. A poor growth potential for both twins in these dyads is a possible explanation. Although these twins had a high cesarean section rate, no severe perinatal anomalies were noted. The large weight differences in the four AGA/LGA dyads were also not reliably predicted by the Δsystolic/diastolic ratio. A likely explanation is that changes in placental resistance are unlikely to be present in this situation.

Comparison with ultrasound findings revealed that the Δsystolic/diastolic ratio was a better predictor of Δbirth weight except for the SGA/SGA dyads. Difficulties in measuring the biparietal diameter and femur length in some dyads was frustrating. Both techniques can also be affected by large shifts in fetal lie. It appears that both the Δsystolic/diastolic and the absolute systolic/diastolic ratios must be carefully determined and followed serially in all cases of twin gestation. We would expect that pulsed Doppler ultrasound may further increase the applicability of this useful variable.

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Umbilical cord size and amniotic fluid volume in prolonged pregnancy

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Expectant management of prolonged pregnancy is predicated on reassuring biophysical testing. However, even the combination of a reactive nonstress test and normal amniotic fluid volume may not prevent subsequent morbidity. To test the hypothesis that diminished cord Wharton's jelly incurs risk of peripartum cord compression in addition to decreased amniotic fluid, 68 patients with confirmed gestational age ≥41 weeks were evaluated prospectively with semiweekly nonstress tests and weekly ultrasound examinations. Amniotic fluid volume was assessed, and umbilical cord diameter was measured and then correlated with the quantity of Wharton's jelly at delivery, determined by cord circumference. A significant correlation between cord circumference and umbilical cord diameter was observed, even in those patients with decreased amniotic fluid volume. Either an amniotic fluid volume <3.8 cm or an umbilical cord diameter <1.6 cm was associated with significant cord compression patterns. Peripartum morbidity was greatest in the presence of a smaller cord and decreased fluid, suggesting a synergism between these two factors for the risk of cord compression in prolonged pregnancy. (Am J Obstet Gynecol 1987;157:716-20.)

Key words: Prolonged pregnancy, Wharton's jelly, amniotic fluid volume

Protocols designed to permit expectant management of prolonged pregnancy have relied on an increased frequency of standard biophysical evaluations. Although semiweekly nonstress tests (NSTs) have been used to ensure fetal well-being, it has been recognized that a reactive NST with variable decelerations can be associated with an unfavorable pregnancy outcome.[1] This finding may relate to the fact that cord compression rather than placental dysfunction accounts for most of the perinatal morbidity in prolonged pregnancy.[2] Furthermore, the risk of cord compression has been related to diminished amniotic fluid volume.[3] To account for this factor, periodic ultrasound assessment of amniotic fluid has been incorporated into most management schemes.[3-6] However, when evaluated prospectively, even the combination of reassuring heart rate testing and adequate amniotic fluid has been associated with significant perinatal morbidity.[7]

Although a normal amount of amniotic fluid has been interpreted as protective against cord compression, the amount of Wharton's jelly may also play a role. Chasnoff and Fletcher[8] provided direct evidence for this role with an in vitro cord model. With artificially knotted cords, they observed an inverse relationship between the amount of Wharton's jelly and the pressure required to perfuse the umbilical vein. As the amount of Wharton's jelly has been shown to decrease with advancing gestational age, we hypothesize that decreased cord Wharton's jelly incurs risk of perinatal cord compression in prolonged gestation. Accordingly, this prospective study was designed with the following objectives: (1) to establish the feasibility of ultrasound measurement of umbilical cord diameter, (2) to relate ultrasound cord diameter to direct measurement of the amount of Wharton's jelly at delivery, (3) to correlate amniotic fluid volume and cord diameter measured by ultrasound with the incidence and severity of variable fetal heart rate decelerations before and during labor, and (4) to determine whether the combination of decreased umbilical cord size and diminished amniotic fluid volume better identifies patients at risk for perinatal morbidity in prolonged pregnancy.

Material and methods

Patients entered into this study were recruited from the antenatal testing unit at Prentice Women's Hospital of Northwestern University from June 1 to Dec. 30, 1986. Informed consent was obtained immediately before biophysical testing. All pregnancies were ≥41 weeks at the time of study entry on the basis of reliable menstrual data, early examination, and/or early ultrasound assessment. After NST completion but before interpretation, ultrasound examination was performed (640 Linear Scanner, Corometrics). Umbilical cord di-

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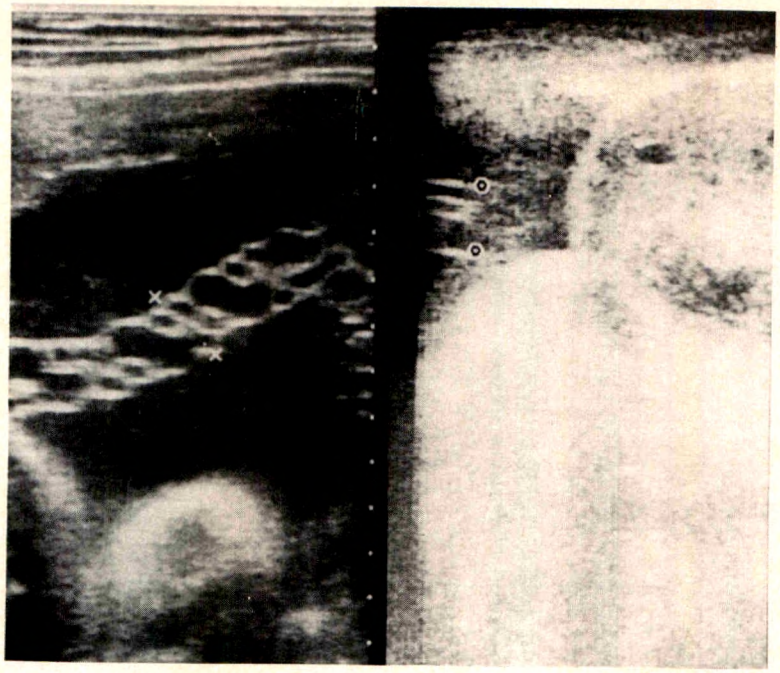


Fig. 1. Illustration of measured cord diameter by ultrasound with both normal and decreased amniotic fluid volume.

ameter was obtained by imaging a longitudinal segment of cord (Fig. 1) and averaging three diameter measurements. Amniotic fluid volume was assessed with the patient in the supine position with lateral uterine displacement. Four uterine quadrants were identified with the midline of the maternal abdomen and the level of the umbilicus used as vertical and horizontal boundaries, respectively. After the largest pocket of fluid in each quadrant was identified, the mean of the three largest vertical measurements was calculated and used to represent amniotic fluid volume. For clinical purposes, amniotic fluid was subjectively categorized as normal, low normal, or oligohydramnios, and this interpretation was made available to the attending physician with the NST results. Umbilical cord diameter and amniotic fluid volume measurements were used only for the purposes of the study. Ultrasound measurements were obtained weekly and NSTs were performed semiweekly until delivery.

To directly measure the quantity of Wharton's jelly at delivery, a segment from the middle third of each cord was rinsed, stripped of blood, and frozen in an occlusive, polyethylene bag at $-20°$ C. Each frozen segment was cross-sectioned at a 90-degree angle at three selected locations, avoiding varicosities. The cut ends were stained with methylene blue and multiple imprints made on paper. Circumference was measured on each imprint in triplicate by means of a digitizer (Model 12405-1, Numonics Corp.). The cord circumference measured in centimeters was derived from the average measurement of six imprints, two from each of the three cross sections.

Antepartum variable decelerations were defined as a decrease in fetal heart rate of $\geq$15 beats/min for at least 15 seconds' duration. The intrapartum fetal heart tracings were interpreted in a standard fashion with the following objective criteria to quantify variable decelerations: (1) Prolonged variable decelerations were defined as >2 minutes in duration with a nadir <90 beats/min whereas severe prolonged variables lasted >4 minutes with a <70 beat/min nadir; (2) total variable deceleration duration was quantified by adding the total deceleration time at <90 beats/min throughout labor (excluding individual decelerations <30 seconds).

Ultrasound and in vitro cord measurements, as well as NST and intrapartum heart rate interpretation, were all performed blinded to each other and to outcome. Data were analyzed with the Statistical Package for Social Sciences (Version X) with χ^2 analysis for discrete variables and t tests (two-tailed) and regression analysis for continuous data.

Results

Sixty-eight of the 72 patients enrolled had complete data obtained and made up the study population. Umbilical cord segments and monitor tracings were not recovered in two patients each. Selected patient characteristics are shown in Table I. The mean gestational age at delivery was 294 days (range 287 to 302 days)

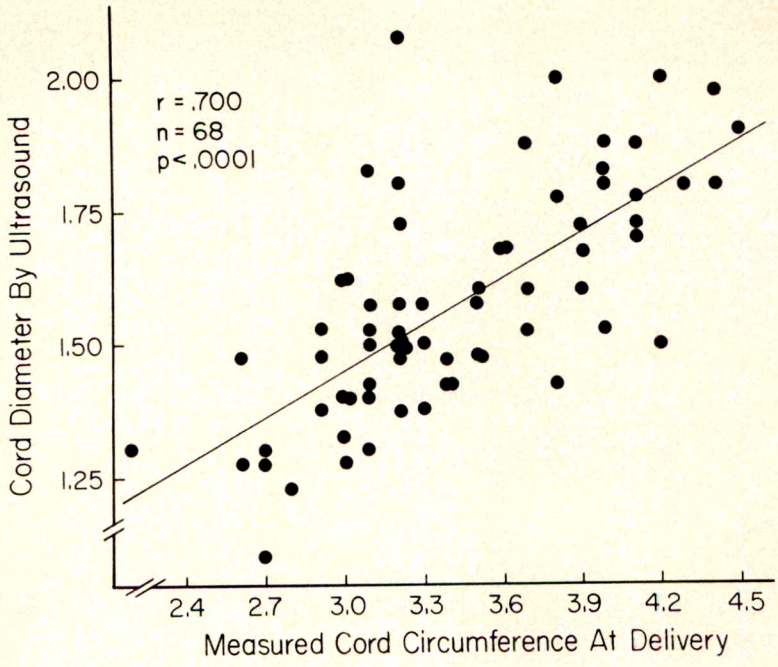


Fig. 2. The relationship of cord diameter by ultrasound to cord circumference at delivery.

Table I. Selected characteristics of 68 patients with prolonged pregnancy

	No.	*%*
Ultrasound examination to delivery interval (days) (mean ± SD)	2.4 ± 2.2	
Duration of intrapartum monitoring (hr) (mean ± SD)	10.5 ± 7.0	
Meconium-stained amniotic fluid	22	(32.3)
Nuchal or shoulder cord at delivery	22	(32.3)
Delivery beyond 42 wk	40	(58.8)
Delivery for fetal heart rate indications		
Cesarean section	10	(14.7)
Operative vaginal	9	(13.2)

and 19 deliveries (27.9%) were performed for fetal heart rate indications. Nine of these were vaginal deliveries (low forceps, N = 6; midforceps, N = 3), in which emergent intervention during the second stage was required as a result of nonremediable, prolonged variable decelerations. Although abnormal cord position at delivery was common in the entire series, there was no association between this finding and either the presence of variable decelerations or fetal heart rate indication for delivery.

Ultrasound measurements revealed a mean amniotic fluid volume of 3.8 cm (range 1.2 to 6.5 cm) and a mean umbilical cord diameter of 1.6 cm (range 1.1 to 2.1 cm). Nineteen and three patients had mean amniotic fluid volume values of <3 and <2 cm, respectively. The mean in vitro cord circumference was 3.4 cm (range 2.2 to 4.5 cm). A significant correlation between in vitro and ultrasound cord measurements was found (Fig. 2). This correlation was present even if analysis was limited to smaller cords (umbilical cord diameter <1.4; N = 19, r = 0.65, p = 0.0027), or those patients with less amniotic fluid (amniotic fluid volume <3.4; N = 27, r = 0.68, p = 0.0001).

Table II demonstrates the impact of amniotic fluid alone on the risk of peripartum cord compression. There were no statistically significant differences when patients were grouped according to amniotic fluid volume being greater or less than 1, 2, or 3 cm. In similar analyses relating umbilical cord diameter to peripartum cord compression, the same trends were seen although significance was not reached. However, there was a significantly smaller mean umbilical cord diameter in the 11 patients with antepartum variable decelerations compared with those without (1.4 versus 1.6; $p < 0.05$). Neither amniotic fluid volume nor umbilical cord diameter related to the incidence of meconium, abnormal

Table II. The relationship between amniotic fluid volume and peripartum fetal heart rate abnormalities

	*Amniotic fluid volume**		
	≥3.8 cm (N = 31)	*<3.8 cm (N = 37)*	
	No. (%)	*No. (%)*	*p Value*
Presence of variable decelerations on NST	4 (12.9)	7 (18.9)	NS
Presence of prolonged variable decelerations†	7 (22.6)	18 (48.6)	<0.05
Total duration of decelerations >110 min†‡	5 (16.1)	15 (40.5)	<0.05
Fetal heart rate indication for delivery	4 (12.9)	15 (40.5)	<0.05
Cord arterial pH at delivery§	7.28 ± 0.08	7.23 ± 0.05	<0.01

*Mean = 3.8 cm.

†See Materials and methods for definitions.

‡Population mean duration of variable decelerations = 110 min.

§Values are mean ± SD.

Table III. Impact of amniotic fluid volume in the presence of smaller or larger umbilical cord diameters

	Smaller cord (N = 40)*		*Larger cord (N = 28)*	
	Lower fluid† (N = 23)	*Higher fluid (N = 17)*	*Lower fluid (N = 14)*	*Higher fluid (N = 14)*
Type of variable deceleration				
During NST	6 (26.1)	3 (17.6)	1 (7.1)	1 (7.1)
Prolonged	13 (56.5)	2 (11.8)	5 (35.7)	5 (35.7)
Severe prolonged	5 (21.7)	1 (5.9)	2 (14.3)	1 (7.1)
Total duration of variable decelerations >110 min	9 (39.1)	3 (17.6)	6 (42.9)	2 (14.3)
FHR indication for delivery	10 (43.5)	4 (23.5)	5 (35.7)	0 (−)
Cord arterial pH‡	7.24 ± 0.05	7.26 ± 0.09	7.22 ± 0.06	7.31 ± 0.05

*Cord size defined as smaller or larger if mean ultrasound diameter < or ≥1.6 cm, respectively.

†Amniotic fluid volume defined as lower or higher if mean vertical pocket < or ≥3.8 cm, respectively.

‡Values are mean ± SD.

cord location, or the interval from rupture of membranes to delivery.

The interaction between amniotic fluid volume and umbilical cord diameter is shown in Table III. The 23 patients with both a smaller umbilical cord diameter and lower amniotic fluid volume had the highest rate of cord compression during labor and were most likely to be delivered for that reason. When these same patients were compared with all others (N = 45), significant increases in the presence of prolonged variables (56.5% versus 26.7%; $p < 0.05$) and fetal heart rate indication for delivery (43.5% versus 20.0%; $p < 0.05$) were also observed. As seen in Table III, although the individual categories have small numbers of patients, a consistent trend of increased morbidity was observed when either fluid volume or cord size was below the population mean. Even the presence of a larger cord was associated with frequent delivery for fetal heart rate indications when amniotic fluid volume was decreased (35.7% versus zero, $p < 0.05$). This same combination was also associated with a lower cord arterial pH at delivery when compared with the 14 patients with both a larger cord and higher fluid volume (7.22 versus 7.31, $p < 0.01$).

The predictive value of amniotic fluid volume, umbilical cord diameter, and the presence of antepartum variable decelerations was assessed with regard to the likelihood of delivery for fetal heart rate abnormalities. In this analysis, sensitivity was lowest with only the presence of variable decelerations on NST used as a discriminator (N = 11, 42%); intermediate with only amniotic fluid volume < 3.8 cm (N = 37, 79%); and highest when a smaller cord and lower fluid volume were present (N = 23; 100%). The combination of all three findings was also 100% sensitive and maximized both specificity (93%) and predictive value (positive = 83%, negative = 100%).

Comment

In this series, ultrasound measurement of the umbilical cord was accomplished without difficulty, even in those cases with decreased amniotic fluid. The cor-

relation between sonographic and in vitro cord measurements demonstrates that the cord diameter observed on ultrasonography reflects the actual quantity of Wharton's jelly, although the former included the contribution of fetal blood.

Instead of prospectively assessing amniotic fluid subjectively (as normal, low, or oligohydramnios) or categorically (as <2 or <1 cm vertical pockets), we chose to use a continuous measure for two reasons: to avoid the unsubstantiated bias that a threshold vertical measurement exists (e.g., 2 cm) above which amniotic fluid is assuredly normal, and (2) to satisfy our expectation that many pregnancies beyond 41 weeks would have relatively diminished fluid that would be inappropriately categorized as normal by traditional definitions. In fact, using our population's mean vertical measurement (3.8 cm) as an arbitrary breakpoint, we observed a significant excess of intrapartum cord compression patterns in patients with amniotic fluid volume below this value, although most of these patients had amniotic fluid volume ≥2 cm. The shortcomings of a categoric definition for decreased amniotic fluid have also been identified by other investigators.[9-11]

To assess the impact of umbilical cord size and amniotic fluid volume together, four sets of circumstances were analyzed. If our hypothesis were true, the most vulnerable combination would be a small cord and low fluid volume (N = 23). Indeed, this setting was found to be associated with the highest risk for significant intrapartum cord compression, although amniotic fluid volume appeared to be the most important predictor of intrapartum morbidity. Using our results to assess the predictive value of these factors separately and in combination, we found that the presence of antepartum variable decelerations, a smaller cord, and lower fluid volume best identified intrapartum morbidity. However, as the number of patients with this combination was small, prospective confirmation of these predictive values is needed.

Previous studies have shown that the water content of Wharton's jelly decreases progressively in late gestation[12] and that water content is significantly correlated with the amount of Wharton's jelly as measured by cord circumference.[13] These findings provoke speculation as to a relationship between water content in both the cord and amniotic fluid. Indeed, a limited correlation between umbilical cord diameter and amniotic fluid volume was observed in this study ($r = 0.25$; $p = 0.038$). However, almost as many larger cords were associated with lower fluid volume (N = 14) as the opposite (N = 17). Therefore it is unlikely that both changes occur simultaneously or at the same rate. Although an interrelation between these two factors is an attractive hypothesis, it cannot be firmly established from these data. A delineation of the biochemical, hormonal, or mechanical factors that regulate the quantities of amniotic fluid and Wharton's jelly may provide a better understanding of these issues.

In summary, antepartum cord assessment appears to be feasible and the results obtained correlate with actual in vitro measurements. We have not identified an absolute umbilical cord diameter or amniotic fluid volume below which risk can be anticipated or above which well-being is assured. However, patients with values below the respective means in this study experienced significant antenatal and intrapartum cord compression, and the combination of umbilical cord size and amniotic fluid quantity appeared to be synergistic with regard to significant fetal heart rate abnormalities in labor. The introduction of umbilical cord measurement as an additional antenatal test during prolonged pregnancy awaits prospective evaluation.

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The origin of increased serum iron in pregnancy-induced hypertension

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Serum iron was measured in 30 patients with pregnancy-induced hypertension and 24 normal pregnant women. The mean iron concentration was significantly higher in the group with pregnancy-induced hypertension (111 ± 26 μg/ml) than in the controls (69 ± 17 μg/ml) ($p < 0.0001$). Readily available laboratory variables were used to determine whether the increased serum iron was the result of (1) hemolysis, (2) hepatocellular injury, or (3) intravascular volume contraction leading to hemoconcentration. It appears that a clinically silent, ongoing hemolytic reaction is responsible for the increase in serum iron seen in patients with pregnancy-induced hypertension. (Am J Obstet Gynecol 1987;157:721-5.)

Key words: Hemolysis, pregnancy-induced hypertension, serum iron

Pregnancy-induced hypertension may be difficult to distinguish from chronic hypertension and other systemic illnesses associated with increased blood pressure. Entman et al.[1] have previously demonstrated that serum iron is increased in patients with pregnancy-induced hypertension. They have proposed that an elevation in serum iron concentration may be a better discriminator than other variables, including uric acid, in differentiating pregnancy-induced hypertension from other forms of hypertension.[1, 2] However, the cause of this increased serum iron is unknown. The body's major stores of iron are located in red blood cells and in the liver.[3] The increase in iron concentration could be the result of destruction of red blood cells or hepatocellular damage. Serum iron might also be elevated as a result of intravascular volume contraction resulting in hemoconcentration. This study was undertaken to determine the cause of this increase in serum iron. We used readily available laboratory variables that could help us to determine whether hemolysis, hepatocellular damage, or hemoconcentration were responsible. An understanding of this phenomenon might provide further insight into the pathophysiology of this disorder.

Methods

We studied 30 patients with pregnancy-induced hypertension and 24 normal patients who came to the Hospital of the University of Pennsylvania between February 1985 and August 1986. Strict criteria were used to diagnose pregnancy-induced hypertension. All patients with pregnancy-induced hypertension (1) had a diastolic blood pressure of at least 90 mm Hg recorded in the left lateral decubitus position on two occasions at least 6 hours apart; (2) had normal blood pressure recordings in the first or second trimester when available; (3) had ≥2+ proteinuria on a catheterized specimen; (4) had a serum uric acid concentration ≥6.0 mg/dl; (5) were primigravidas; and (6) had no history or laboratory evidence of hypertension, renal disease, or collagen vascular disease. Only five patients with pregnancy-induced hypertension had sustained diastolic blood pressures ≥110 mm Hg.

Control patients (1) were primigravidas; (2) were normotensive throughout their prenatal course and the puerperium; (3) had trace or less proteinuria on a clean-catch specimen; (4) had a serum uric acid concentration ≤4.7 mg/dl; and (5) had no history or laboratory evidence of a systemic disease.

All patients were studied, with the use of standard laboratory techniques, for evidence of hemolysis (haptoglobin, plasma-free hemoglobin, and reticulocyte count); hepatocellular damage causing release of iron (alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, or serum ferritin); and intravascular volume contraction leading to hemoconcentration (hemoglobin and blood urea nitrogen:creatinine ratio). Two other measured variables, total bilirubin and lactate dehydrogenase (LDH), could represent either hemolysis or hepatocellular damage. Approximately 20 ml of blood was obtained by venipuncture in test tubes containing the appropriate anticoagulant or preservative for the tests being performed.

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Table I. Characteristics of patients studied

	Pregnancy-induced hypertension (*n* = 30)	*Normal* (*n* = 24)
Mean age (yr)	21.8	22.4
Age range (yr)	16-27	19-27
Mean gestation (wk)	35.8	37.5
Gestation range (wk)	28-40	34-42
Black/white	23/7	19/5
Primigravidas	All	All
No prenatal care	5	0

All samples were processed within 4 hours of collection. All blood was drawn before the onset of labor and before the patients with pregnancy-induced hypertension received magnesium sulfate. Blood urea nitrogen, serum creatinine, gamma glutamyl transferase, and total bilirubin determinations were made with a commercial kit for the Ektachem-700 (Kodak) analyzer. Aspartate aminotransferase, alanine aminotransferase, and LDH determinations were made with the use of a kit for the Encore (Baker) analyzer. Haptoglobin was determined with a commercial kit for the Cobas Bioanalyzer (Roche). Serum iron was assayed by the controlled potential colorimetry method with the Ferrochem 3050 Iron and TIBC analyzer (Environmental Science Associates). Plasma hemoglobin was determined by the ortho-tolidine, acetic acid method and read in a spectrophotometer at 625 nm.[4] Serum ferritin was analyzed by the Ferrizyme EIA kit with the Quantum II analyzer. Reticulocytes were stained with a vital stain and counted manually.

Statistical analysis was performed by means of the Statworks program for the Apple Macintosh Plus computer. Means were compared with the two-tailed Student *t* test. Correlations between serum iron and other variables were determined with the use of linear regression. We also used multiple linear regression to determine the proportion of serum iron attributable to the subgroups of hemolysis, hepatocellular injury, and intravascular volume contraction.

Results

The groups were closely matched for age, race, and gravidity (Table I). The average length of gestation was shorter in the group with pregnancy-induced hypertension than in the control group (35.0 ± 3.7 weeks versus 37.5 ± 2.5 weeks) ($p = 0.07$, Student's *t* test). Whereas five of the patients with pregnancy-induced hypertension had not sought prenatal care, all of the control patients had been under the care of a physician during pregnancy.

Only five patients met blood pressure criteria for severe pregnancy-induced hypertension (persistent diastolic blood pressure ≥110 mm Hg). Because serum iron levels did not correlate with the degree of hypertension or proteinuria, we did not distinguish between patients with mild and severe pregnancy-induced hypertension in this study. None of the patients studied became eclamptic.

We found that patients with pregnancy-induced hypertension have a higher serum iron concentration (111 ± 26 μg/dl) when compared with normal control pregnancies (69 ± 17 μg/dl) ($p < 0.0001$). This observation confirms the findings of Entman et al.[1] The mean of each variable studied in patients with and without pregnancy-induced hypertension and the statistical significance of the differences are displayed in Table II. Using the technique of linear regression, we determined the correlation between serum iron concentrations and the levels of the various variables studied in patients with pregnancy-induced hypertension. The results of linear regression analysis are shown in Table III.

The strongest correlations were between serum iron and LDH, plasma hemoglobin, haptoglobin, and the reticulocyte count. These correlations are graphically depicted in Fig. 1. There was also a strong correlation between total bilirubin and serum iron concentrations (coefficient of correlation = 0.67). Each patient had a direct bilirubin determination performed. Only two patients had concentrations exceeding 0.2 mg/dl, one being 0.3 mg/dl and the other 0.4 mg/dl.

Parameters of hepatocellular damage correlated poorly with serum iron levels (Table III). The relationships between serum iron and the indices of volume contraction were not significant.

We then used multiple linear regression to determine the proportion of the serum iron in patients with pregnancy-induced hypertension that could be attributed to hemolysis, hepatocellular damage, and volume contraction. The results, shown in Table IV, clearly demonstrate that hemolysis is the largest contributor to the increase in serum iron seen in the patients with pregnancy-induced hypertension we studied.

Comment

We have confirmed the observation that serum iron is increased in patients with pregnancy-induced hypertension.[1] A variety of laboratory parameters and statistical analyses suggest that this increase in iron arises from a mild, ongoing intravascular hemolysis. We did not separate patients with mild and severe pregnancy-induced hypertension because serum iron levels did not correlate with severity of disease. However, only five patients met blood pressure criteria for severe pregnancy-induced hypertension, and only four of them had a platelet count below 150,000/mm³. None

Table II. Comparison of parameters studied in patients with and without pregnancy-induced hypertension

	Normal (n = 24)	*Pregnancy-induced hypertension (n = 30)*	*p Value**
Serum iron (μg/dl)	69 ± 17	111 ± 26	<0.0001
Hemolysis			
Plasma hemoglobin (mg/dl)	0.4 ± 0.3	3.5 ± 1.7	<0.0001
Haptoglobin (mg/dl)	120 ± 30	70 ± 25	<0.0001
Reticulocyte (%)	1.3 ± 0.8	2.3 ± 0.8	<0.0001
Hemolysis or liver			
LDH (IU/L)	210 ± 35	315 ± 95	<0.0001
Total bilirubin (mg/dl)	0.6 ± 0.2	1.1 ± 0.2	<0.0001
Liver			
ALT (IU/L)	14 ± 7	17 ± 9	0.413
AST (IU/L)	15 ± 5	16 ± 11	0.672
GGT (IU/L)	18 ± 4	13 ± 7	0.004
Ferritin (ng/ml)	25 ± 11	30 ± 11	0.078
Volume contraction			
Hemoglobin (gm/dl)	11.1 ± 1.2	11.2 ± 1.1	0.653
BUN/creatinine	10.7 ± 3.5	12.5 ± 3.5	0.68

LDH = Lactate dehydrogenase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamm glutamyl transferase; BUN = blood urea nitrogen.

*Two-tailed *t* test.

Table III. Correlation between serum iron and parameters studied in 30 patients with pregnancy-induced hypertension

	r	r^2	*p Value*
Hemolysis			
Plasma hemoglobin	0.83	0.67	<0.0001
Haptoglobin	0.79	0.61	<0.0001
Reticulocyte	0.75	0.54	<0.0001
Hemolysis or liver			
LDH	0.84	0.70	<0.0001
Total bilirubin	0.67	0.42	<0.0001
Liver			
ALT	0.48	0.20	0.007
AST	0.47	0.19	0.008
GGT	0.23	0.017	0.23
Ferritin	0.20	0.009	0.027
Volume contraction			
Hemoglobin	0.24	0.023	0.21
BUN/creatinine	0.23	0.021	0.22

For abbreviations see Table II.

Multiple linear regression was used for analysis.

Table IV. Proportion of elevated serum iron attributable to grouped parameters

Group	*Attributable portion (r^2)**	*p Value*
Hemolysis	0.848	<0.0001
Hepatocellular damage	0.243	0.026
Hemoconcentration	0.011	0.341

Group parameters as defined in text.

*Multiple linear regression (adjusted r^2).

had a depressed fibrinogen level. Therefore frank hemolysis, as seen with the fulminant disseminated intravascular coagulopathy that may complicate severe pregnancy-induced hypertension, is unlikely to have contributed significantly to this process.

Haptoglobin, plasma hemoglobin, and reticulocytosis are solely indicative of hemolysis. However, LDH can originate from red blood cells or the liver. We did not have the ability to distinguish between these isoenzymes. No other parameter of liver function correlated with serum iron, so we believe the difference in LDH was probably a result of hemolysis.

Total bilirubin can also be the result of either hemolysis or hepatocellular injury. With hemolysis, the indirect component of bilirubin is generally elevated because it saturates the conjugating enzyme mechanism. With liver dysfunction, the direct component is often elevated because conjugated bilirubin cannot be excreted. Because the major portion of the bilirubin was unconjugated in the patients we studied, we believe that this represents hemolysis.

Serum ferritin, a protein that complexes iron, is the major storage form of iron found in the liver, spleen, and bone marrow.[5] Ferritin is an accurate reflection of the body's iron stores unless there is hepatocellular damage with release of stored ferritin into the circulation. Although Entman et al.[6] noted an increase in serum ferritin in patients with pregnancy-induced hypertension, it was not sufficient to account for their

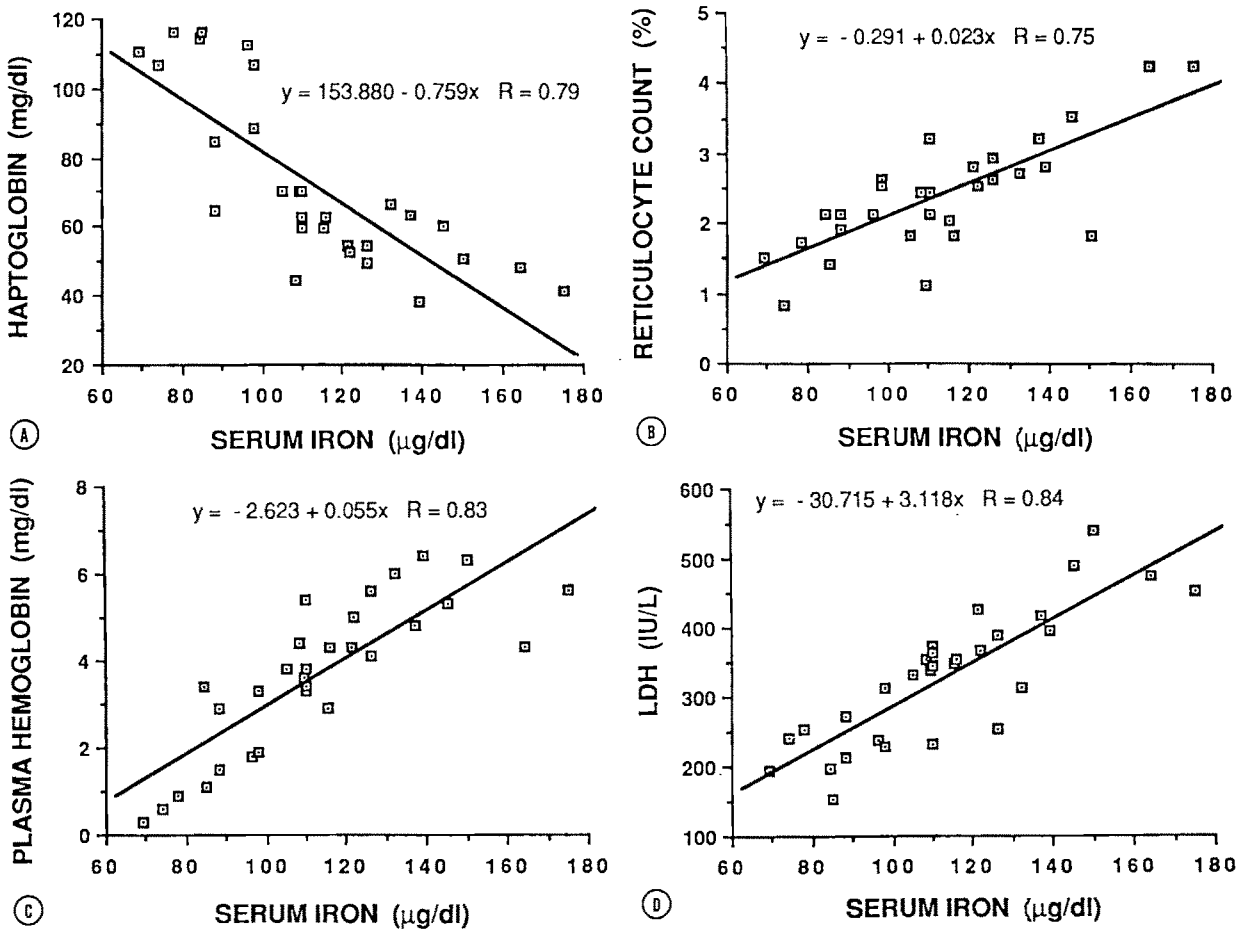


Fig. 1. Correlation between serum iron concentration and parameters of hemolysis in 30 patients with pregnancy-induced hypertension. Linear regression was used to determine correlations of coefficient between the concentration of serum iron and the level of haptoglobin (**A**), reticulocyte count (**B**), plasma-free hemoglobin (**C**), and lactate dehydrogenase (**D**).

observed increase in serum iron. Our patients did not show this increase in serum ferritin. In our series, the concentrations were essentially the same in patients with and without pregnancy-induced hypertension (Table II).

We cannot fully explain this conflict between our data and those of Entman et al.[6] Most of our patients were followed up closely in prenatal clinics and we readily admitted patients who were showing early signs of pregnancy-induced hypertension. Perhaps a difference in the severity and duration of disease is in part responsible for these discrepant findings. In addition, it is possible that the prepregnancy iron stores were different in patients in the two studies, accounting for part of this difference. We did confirm a poor correlation between serum iron and serum ferritin concentrations.

Volume contraction is seen quite frequently in patients with severe pregnancy-induced hypertension.[7, 8] Goodlin et al.[9] have shown that the ratio of blood urea nitrogen:creatinine is an indicator of intravascular volume contraction. We chose both this parameter and hemoglobin concentration as indicators of hemoconcentration to assess whether this phenomenon could account for the increase in serum iron seen in pregnancy-induced hypertension. Six patients with pregnancy-induced hypertension and three control patients exhibited intravascular volume contraction as reflected by a blood urea nitrogen:creatinine ratio >15. Linear regression did not show a significant correlation between parameters of volume contraction and serum iron concentration in the 30 patients with pregnancy-induced hypertension we studied (Table III). It is likely that volume contraction was suggested in only 20% of our patients with pregnancy-induced hypertension because most were diagnosed early in the course of their disease. In addition, it is possible that the parameters we studied are not so sensitive as more invasive techniques for determining plasma volume. Nonetheless, it does not appear that volume contraction accounts for

a significant portion of the increase in serum iron seen in our patients.

Hemolysis and disseminated intravascular coagulation have long been recognized as complications of severe pregnancy-induced hypertension.[10, 11] They are necessary components of the HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome.[12] Our study demonstrates that mild hemolysis is part of the constellation of signs and symptoms constituting pregnancy-induced hypertension and is not limited to severe disease. Although laboratory evidence of hemolysis correlated well with serum iron levels, these parameters often remained within the normal limits for our clinical laboratory. Therefore, we believe that these laboratory values cannot be used alone to establish a diagnosis of pregnancy-induced hypertension. However, these parameters can be used in conjunction with other clinical and laboratory studies to help with diagnoses in difficult cases.

In summary, our study provides new insight into the pathophysiology of pregnancy-induced hypertension. The elevated serum iron levels observed in many patients with this disorder are derived in large part from hemolysis. The cause of this hemolytic reaction is unknown. It may be caused by physical destruction of red blood cells as a result of intense vasospasm or abnormal endothelial cell–erythrocyte interactions. Conversely, it may be due to immune destruction of red blood cells. The cause of this hemolysis and the role it plays in the pathogenesis and clinical expression of pregnancy-induced hypertension require further investigation.

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The effects of long- and short-term maternal caffeine ingestion on human fetal breathing and body movements in term gestations

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The possible effects of long- and short-term maternal ingestion of caffeine during normal human pregnancy on the breathing and body movements of third-trimester fetuses were studied in 14 patients, selected by a dietary questionnaire, and divided into two equal groups: high consumers (>500 mg/day) (group 1) and low consumers (<250 mg/day) (group 2). All mothers followed a standard study protocol and underwent overnight fasting; studies began with a 30-minute control period, followed by oral administration of 200 mg caffeine, and a 180-minute subsequent observation period with continued maternal fasting. Blood samples for glucose and caffeine were obtained every 30 minutes and continuous recording of fetal breathing and body movements were entered on a microcomputer for off-line analysis. The two groups were similar for all obstetric outcome features. Plasma glucose levels were similar and constant in both groups whereas caffeine levels increased significantly at 60 minutes after administration; mean plasma caffeine levels were significantly higher in group 1 than in group 2 at all intervals. Fetal breathing rates and body movement incidences were similar in both groups before and after caffeine administration. Fetal breathing movement incidence decreased significantly in group 2 but was sustained at baseline levels in group 1 throughout the study. High long-term ingestion of caffeine during pregnancy is associated with higher maternal plasma caffeine levels and fetal breathing activity when compared with low caffeine ingestion. Short-term administration of 200 mg caffeine does not appear to have a significant physiologic impact on these activities. (AM J OBSTET GYNECOL 1987;157:726-9.)

Key words: Caffeine, fetal breathing and body movements, term gestation

Caffeine, a central nervous system stimulant, is consumed by an estimated 95% of American women during their pregnancies.[1] Although its metabolism and physiologic effects have been extensively studied in mothers and infants, little is known about its effects on the human fetus. Maternal caffeine clearance decreases near term.[2,3] Caffeine and theophylline, a caffeine precursor, are rapidly transferred across the placenta[4] and alter both sleep states and cardiorespiratory activity parameters in neonates.[5,6] Both drugs have been used clinically in the treatment of prematurity-related apnea.[7,8] Direct infusion of caffeine in chronic lamb preparations have shown a transient but significant increase in fetal breathing activity.[9] These observations led us to perform the present study to determine (1) whether differences in long-term levels of caffeine consumption during human pregnancy resulted in observable differences in fetal breathing or body movements; and (2) whether short-term administration of oral caffeine to normal pregnant women had a physiologic impact on these fetal activities.

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Material and methods

Fourteen normal pregnant women in the third trimester of pregnancy were enrolled from the Obstetrical Clinic of the Medical College of Georgia. After informed consent was obtained to participate in a study protocol, approved by the institutional Human Assurance Committee, a detailed dietary history for the current pregnancy was obtained and the patients were then classified into either a high (>500 mg/day) caffeine-consuming group (group 1) or a low (<250 mg/day) group (group 2). All patients had normal prenatal courses, well-established gestational dates by menstrual history and ultrasound mensuration, normal responses to glucose-loading tests, and singleton fetuses that were normal for gestational age.

Before each study, a period of fasting was begun at 10:00 PM of the preceding evening. Patients came to the fetal study unit at 8:00 AM the next day. After the patient was placed in bed in a semi-Fowler position with

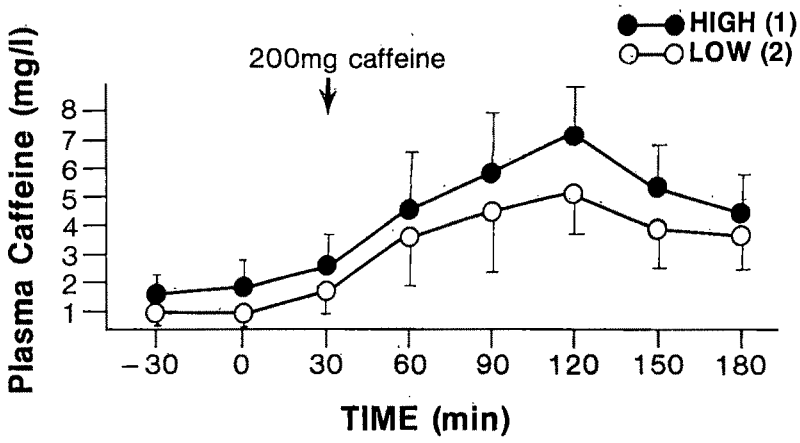


Fig. 1. Mean maternal serum caffeine levels for high caffeine consumers *(closed circles)* and low caffeine consumers *(open circles)*. *Bars* represent standard deviations.

a slight lateral hip displacement, a heparin-lock intravenous catheter was placed in a peripheral arm vein. Blood samples for blood glucose and caffeine levels were obtained at the beginning of each study and at 30-minute intervals until its conclusion 210 minutes later. These samples were divided with blood glucose determined by means of a BG Accuchek analyzer (Bochinger Mannheim Diagnostics, Inc.) and serum caffeine determined after mobile phase solution preparation, processing the extracted serum on reverse-phase column with a Beckman Solvent Delivery 421 module with a 160 absorbance detector and a Hewlett-Packard 3390 integrator.

Studies began with the placement of an ultrasound transducer (ATL 4000/L) on the mother's abdomen, aligned with a sagittal view of the fetal thorax and abdomen. Three observers, two of whom were ignorant of the patient's dietary history, participated in fetal observations. The order of observer participation varied randomly with each study. Fetal breathing movements were defined as the paradoxical movements of the thorax and abdomen about the xiphoid, apnea as the absence of breathing or body movements for more than 6 seconds, and body movements as axial displacement sufficient to obscure fetal breathing movements. Breathing and body movements were entered on-line, via an event marker, to an IBM X/T microcomputer, 384k RAM, with a previously validated software program[10] for acquisition and analysis. Maternal fasting was maintained throughout each study period, and at 30 minutes of observation, each mother was given an oral dose of 200 mg caffeine, with continued observation of the fetus for 180 minutes. This dose of caffeine is equivalent to two cups of regular coffee and is the lowest at which sleep disturbances have been observed in adults.[11]

Data analyzed included mean blood glucose and serum caffeine levels, mean time spent in breathing and body movements, mean breath rate, fail time, gestational age at testing and delivery, fetal weight, and 5-minute Apgar scores. Statistical comparisons used paired *t* tests or analysis of variance with repeated measures, as indicated in the text or tables.

Table I. Obstetric data for study patients

	Group 1 (n = 7)	*Group 2 (n = 7)*
Gestational age at study (wk)	38.0 ± 0.0	37.5 ± 1.8
Gestational age at delivery (wk)	40.5 ± 1.1	39.9 ± 1.0
Birth weight (gm)	3412 ± 369	3342 ± 339
5-min Apgar score	9.1 ± 0.9	9.0 ± 0.8

Results expressed as mean ± SD.

Results

Obstetric data and pregnancy outcome. The group means and standard deviations for gestational age at the time of study and delivery, birth weight, and 5-minute Apgar score are presented in Table I. There were no significant differences between the groups for any of these factors. All infants were born in good condition, were appropriate for gestational age, and without significant anomalies. No mothers had any peripartum complications.

Maternal blood glucose and serum caffeine levels. From the results of the maternal dietary questionnaire, group 1 had a significantly higher mean daily intake of caffeine (863 ± 294 mg/day [mean ± standard deviation]) than group 2 (100 ± 42 mg/day) ($p < 0.001$, *t* test). Fig. 1 illustrates the course of mean maternal serum caffeine levels. Group 1 began at and maintained significantly higher serum caffeine levels than group 2

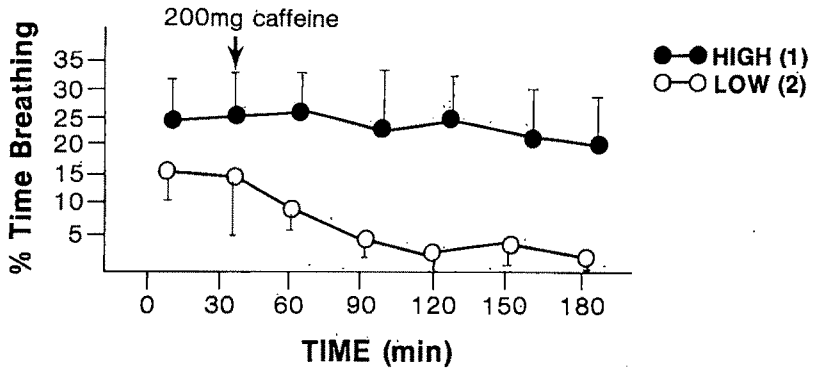


Fig. 2. Mean percentage of time spent in fetal breathing activity for high caffeine consumers *(closed circles)* and low caffeine consumers *(open circles)*. *Bars* represent standard deviations.

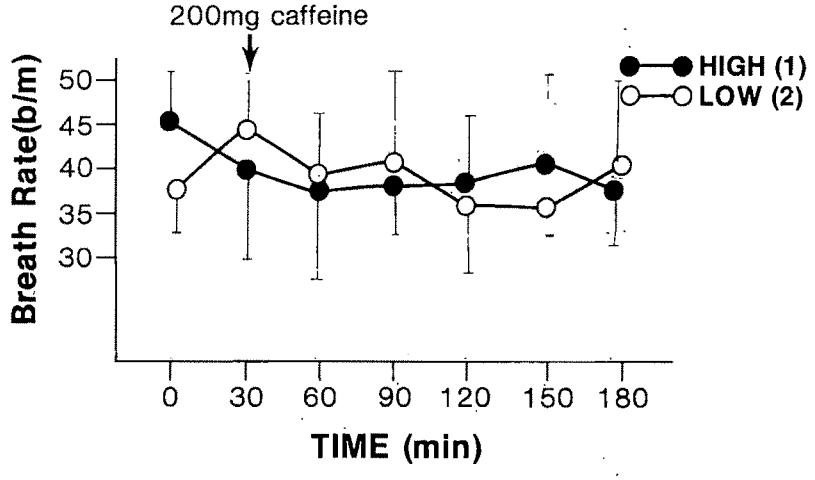


Fig. 3. Mean 30-minute fetal breathing rates for high caffeine consumers *(closed circles)* and low caffeine consumers *(open circles)*. *Bars* represent standard deviations.

at all study intervals. Serum caffeine peaked in both groups at 60 minutes after maternal administration of the 200 mg loading dose and remained significantly higher than baseline values for the remainder of the studies. Mean maternal blood glucose levels (not illustrated) were 78.5 ± 2.5 (SD) mg/dl and 80 ± 2.1 (SD) mg/dl for groups 1 and 2, respectively, and did not vary significantly throughout the observation period.

Fetal breathing activity. Figs. 2 and 3 show mean 30-minute percentages of time spent in breathing and breath rates, respectively, for groups 1 and 2. Although neither group had a significant increase in percentage of time spent breathing, after maternal caffeine administration, the mean 30-minute breathing activity was significantly greater ($p < 0.01$, repeated measures of variance) in the high caffeine-consuming group 60 minutes after caffeine loading and remained so throughout the studies. Mean 30-minute breathing rates were similar for both groups during the studies and did not change significantly from baseline levels.

Fetal body movements. Fig. 4 shows the percentage of time spent in body movements for groups 1 and 2. Although there was a significant decline in body movement activity 90 minutes after caffeine loading in both groups ($p < 0.01$, paired t test), there was no significant trend with time in either group when compared with baseline values, which were similar between the groups.

Comment

Caffeine has been shown to stimulate respiration in premature infants[6] and alters their time spent in rapid-eye-movement sleep.[5] The limited animal data available suggest that bolus administration of caffeine directly to chronically instrumented fetuses results in an abrupt and significant increase in fetal breathing activity.[9] Our present study was an initial attempt to determine both the potential long- and short-term effects on fetal breathing activity associated with maternal caffeine ingestion and thus has no previous human fetal observations with which to compare its findings.

Insufficient numbers of paired maternal and fetal blood samples were available to determine their correlation or whether they were significantly higher for infants of mothers who were high caffeine consumers

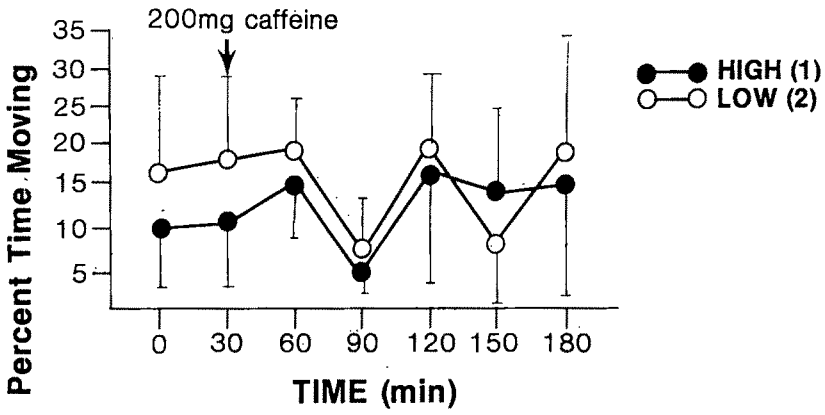


Fig. 4. Mean percentage of time spent in fetal body movement for high caffeine consumers *(closed circles)* and low caffeine consumers *(open circles)*. *Bars* represent standard deviations.

(group 1). However, previous reports suggest that this is quite likely due to the delayed maternal and fetal clearance of caffeine near term.[2, 12] The mechanism by which caffeine exerts its effect on respiratory regulation is still uncertain, although it has been postulated that it enhances respiratory center sensitivity to carbon dioxide,[13] a potent stimulator of in utero breathing activity.[14] The fetal breathing movement percentages, observed in cases where high maternal consumption of caffeine had been present during pregnancy, are higher than would be expected in a previously studied group of normal fasting patients at term.[15] This, in association with the higher mean serum caffeine levels observed in these mothers at the start of the study period, suggests a possible influence for long-term caffeine ingestion during pregnancy on the long-term modulation of fetal breathing activity. It should be noted as well that the breathing activity patterns of fetuses whose mothers were low consumers of caffeine very closely resembled those of normal fasting patients.[15] The absence of significant changes in time occupied by fetal body movements in either group suggests that, if long-term caffeine ingestion plays a role in fetal developmental physiology, it is a somewhat selective one. The decrease in fetal body movement frequency, noted transiently in both study groups, 90 minutes after acute maternal caffeine ingestion, more likely represents a normal cyclic variation in this activity.

Safe levels of maternal caffeine consumption during pregnancy remain to be established. However, the apparent lack of effect on essential fetal physiologic activities, after maternal administration of 200 mg caffeine and in mothers with a history of low daily intake of caffeine during pregnancy, suggests that such a level of caffeine usage may have little influence on fetal behavior in the third trimester of pregnancy. Further studies would be needed to confirm these observations and to establish whether an upper limit of caffeine intake exists beyond which significant fetal biophysical effects might be consistently present.

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Leukocyte esterase activity in human amniotic fluid for the rapid detection of chorioamnionitis

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Chorioamnionitis plays an important role in perinatal morbidity and mortality. Fast and accurate diagnosis poses a major problem. A prospective study was performed to assess the value of positive leukocyte esterase test (Chemstrip 9) for the diagnosis of chorioamnionitis during labor. We evaluated 21 patients with chorioamnionitis in labor at term and used 21 matched control subjects. The sensitivity and specificity of leukocyte esterase activity were compared with those of amniotic fluid cultures, Gram stains, maternal pyrexia and leukocytosis, and fetal tachycardia. The sensitivity in diagnosing chorioamnionitis was 91% and the specificity was 95%. The use of this test strip could provide a rapid, inexpensive screening test for chorioamnionitis. (Am J Obstet Gynecol 1987;157:730-2.)

Key words: Leukocyte esterase, chorioamnionitis

Chorioamnionitis plays a major role in perinatal morbidity and mortality. One of the basic problems in diagnosing chorioamnionitis is the lack of uniformity and accuracy of the criteria on which the diagnosis is made. Microscopic examination of the amniotic fluid for the presence of leukocytes and bacteria is an important part of the evaluation for chorioamnionitis. Neutrophilic leukocytes contain several esterases that are not present in serum, urine, or other body fluids.[1-3] Recently, Chemstrip 9 (Biodynamics Division of Boehring Mannheim Corporation, Indianapolis, Indiana) dipsticks were released for use in the detection of leukocytes. The test strip detects the presence of a leukocyte esterase that catalyzes the hydrolysis of an indoxyl carboxylic acid ester to indoxyl. The indoxyl reacts with a diazonium salt to produce a purple color.[3] The esterase activity measured by the assay does not require intact cells and is not affected by changes in the pH, cell, protein, or bacterial content of the fluid.[1] Limitations of the test include interfering substances such as oxidizing or reducing agents, formalin, and some drugs (cephalexin, gentamicin, nitrofurantoin, and phenpyrazine).

We have recently reported on the reliability of leukocyte esterase screening for the detection of urinary and lower genital tract infections in obstetrics patients.[4] In the present study we assessed the feasibility of rapid screening for the presence of leukocytes in infected amniotic fluid by use of Chemstrip 9 dipsticks to detect leukocyte esterase activity.

Material and methods

After informed consent was obtained, amniotic fluid specimens obtained aseptically were collected via aspiration through intrauterine pressure catheters from 21 patients in labor at term. All patients had required placement of the intrauterine pressure catheter for obstetric indications. Each patient had clinical evidence of chorioamnionitis on the basis of the presence of at least two of the standard clinical criteria used for its diagnosis, for example, maternal pyrexia, leukocytosis, uterine tenderness, or fetal tachycardia. Each patient with intra-amniotic infection was matched with a control patient for gestational age, labor, time from rupture of the membranes to delivery, and time from rupture of the membranes to collection of the amniotic fluid. The initial 10 ml of each sample of aspirated fluid was discarded to avoid possible contamination from vaginal flora. Each amniotic fluid sample was divided into two equal parts, one of which was transported in sterile capped syringes to the laboratory for aerobic and anaerobic cultures and the other of which was used for the various tests mentioned here. On arrival in the laboratory, the fluid was immediately inoculated into Bactec (Johnston Laboratories, Towson, Maryland) aerobic and anaerobic culture bottles in preparation for obtaining the cultures. Leukocyte esterase activity was determined by dipping Chemstrip 9 dipstick in the amniotic fluid for 1 second. The strip was then withdrawn over the specimen cup rim to remove excess fluid and

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The opinions and assertions contained herein are those of the authors and are not to be construed as official or those representing the United States Navy or the Department of Defense.

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Table I. Results of parameters used to detect chorioamnionitis

	Chorioamnionitis		*Control*	
	Pos.	*Neg.*	*Pos.*	*Neg.*
Amniotic fluid				
Cultures	18	3	2	19
Gram stains	13	8	4	17
Maternal pyrexia	13	8	2	19
Maternal leukocytosis	14	7	3	18
Fetal tachycardia	13	8	4	17
Leukocyte esterase	19	2	1	20

Table II. Reliability of parameters used to detect chorioamnionitis

	Sensitivity	*Specificity*	*Positive predictive value*	*Negative predictive value*
Amniotic fluid				
Cultures	86	91	90	86
Gram stains	62	81	77	68
Maternal pyrexia	62	91	87	70
Maternal leukocytosis	67	86	82	72
Fetal tachycardia	62	81	77	68
Leukocyte esterase	91	95	95	91

incubated at room temperature for 1 minute. The color reaction was compared with the color chart, and the results were immediately recorded. The leukocyte esterase results were retrospectively compared with the results of the following parameters: amniotic fluid cultures and Gram stains, maternal leukocytosis and pyrexia, and fetal tachycardia. The sensitivity, specificity, and predictive indices for these parameters were calculated.

A positive leukocyte esterase test was defined as a color reaction consistent with a 1+ reading on the accompanying color chart. Maternal pyrexia was defined as a temperature $\geq 37.8°$ C. Maternal leukocytosis was defined as a white blood cell count $\geq 12{,}000/mm^3$. A positive culture was defined as the presence of $\geq 10^4$ colony-forming units (cfu) of one clearly predominant organism per milliliter. A positive Gram stain was defined as the presence of any bacteria per oil immersion field (magnification $\times 100$). Fetal tachycardia was defined as a heart rate >160 bpm.

Results

Table I represents the summation of the various parameters studied in both the chorioamnionitis and the control groups. In the group with chorioamnionitis, 18 of the 21 patients had positive amniotic fluid cultures with organisms $>10^4$ cfu/ml. A single organism was identified in 15 of these specimens. *Escherichia coli* was the predominant clinical isolate in 60% (nine of 15) followed by *Staphylococcus epidermidis* in 20% (three of 15) and group B streptococci in 20% (three of 15). Three of the 21 patients had positive amniotic fluid cultures with more than one organism identified. One patient had *E. coli* and *S. epidermidis* and two had Enterobacteriaceae with group B streptococci. Thirteen patients demonstrated positive Gram stains. The organisms identified were *E. coli* in seven, *S. epidermidis* in two, and group B streptococci in four of the specimens. Leukocyte esterase activity was positive in 19 of the 21 patients in this group. Eighteen of them also had positive amniotic fluid cultures with organisms $>10^4$ cfu/ml. The remaining patient had a growth of only 10^2 cfu/ml on culture. Thirteen of the 18 patients with positive leukocyte esterase activity also showed evidence of bacteria on Gram stain. However, six of the 13 patients with positive leukocyte esterase activity had negative Gram stains but had varying numbers of white blood cells on the slides. In the control group, the two patients with positive amniotic fluid cultures each grew a single organism (*E. coli* and *Enterobacter aerogenes*). Four patients had positive Gram stains (two with *E. coli* and two with *S. epidermidis*) but none developed signs of infection. One patient in this group had positive test results for leukocyte esterase. She had a positive amniotic fluid culture and a positive Gram stain with *E. coli* as the isolate. She also did not demonstrate clinical evidence of chorioamnionitis.

Table II shows the sensitivity, specificity, and predictive values of the various parameters used in the comparison with leukocyte esterase activity. In our study,

positive leukocyte esterase activity had a 91% sensitivity and a 95% specificity whereas positive amniotic fluid cultures only showed 86% sensitivity and 91% specificity.

Comment

Acute chorioamnionitis usually complicates premature or prolonged rupture of the membranes and remains a potentially lethal infection for mother and baby. Despite the seriousness of this condition, there are major problems associated with its diagnosis. At present it is impossible to determine what constitutes an optimal diagnostic finding. When Smith et al.[5] reviewed the problem of perinatal infection several years ago, they concluded that fever and persistent tachycardia were the main criteria for diagnosing fetal infection. Larsen et al.[6] reported that the presence of polymorphonuclear leukocytes in the amniotic fluid reliably predicted clinical intrauterine infection (presence of polymorphonuclear leukocytes denoting a 100% infection rate), but when a positive bacterial culture was the only finding the infection rate was the same as for patients with neither polymorphonuclear leukocytes nor bacteria. Other reports in the literature show poor correlation of various parameters for the prediction of infection. Listwa et al.[7] studied samples from 95 internally monitored patients and found that the presence of polymorphonuclear leukocytes or bacteria did not predict infection, nor did their absence assure that infection would not occur. Gibbs et al.[8] analyzed amniotic fluid from 35 patients with rupture of the membranes before operative delivery. The presence of more than one polymorphonuclear leukocyte per oil immersion field did not identify those patients at high risk for infection. Thus, for patients suspected to have chorioamnionitis, analysis of amniotic fluid for white blood cells or bacteria seems of little predictive or diagnostic value. These observations are similar to our results where amniotic fluid Gram stains only showed a 62% sensitivity and an 81% specificity and maternal leukocytosis showed a sensitivity and a specificity of 67% and 86%, respectively. Bobitt and Ledger[9] evaluated quantitative amniotic fluid cultures from 28 high-risk laboring patients. Those with colony counts $<10^3$ organisms per milliliter showed no subsequent clinical infection, whereas 56% of the patients with counts $>10^3$ cfu/ml became clinically infected. In our study, amniotic fluid cultures had a sensitivity and specificity of only 86% and 91%, respectively. This may be explained by the fact that the presence of bacteria in amniotic fluid (and their subsequent isolation on culture) does not always correlate with clinical evidence of chorioamnionitis. Also, the sensitivity and specificity of leukocyte esterase activity appear to be superior to those of amniotic fluid cultures for detecting chorioamnionitis because of the limitations of the laboratory facilities that provide the results, specifically in the reporting of positive anaerobic cultures.

Attempts to improve our ability to diagnose intrapartum infection have met with little success. In order to try and identify an optimal screening test for chorioamnionitis, we present our findings in which the use of leukocyte esterase activity allows the detection of infection even in the absence of significant numbers ($>10^4$ cfu/ml) of organisms on culture. Moreover, the use of amniotic fluid cultures is costly and time consuming, whereas leukocyte esterase activity detection is simple, rapid, inexpensive, and easily reproducible. Also, its high sensitivity and specificity suggest that leukocyte esterase–negative amniotic fluid specimens could be eliminated from further testing (for example, cultures and Gram stains). However, the use of this test as a screen to eliminate additional testing or as a preliminary finding on which to base management decisions needs further evaluation in various patient populations and at varying gestational ages.

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Changes in cyclic adenosine monophosphate–phosphodiesterase activity in nonpregnant and pregnant human myometrium

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Cyclic adenosine monophosphate–phosphodiesterase is the enzyme responsible for cyclic adenosine monophosphate degradation. We investigated the kinetic behavior of this enzyme in the myometrium of women who were nonpregnant, pregnant at term not in labor, and pregnant at term in active labor. Phosphodiesterase activity was measured in the 100,000 g supernatant by the two-step isotopic procedure. The Km (Michaelis constant) value remains essentially unchanged from the nonpregnant to the pregnant state and subsequent labor in both the low and the high affinity enzymes. During pregnancy the V max (maximum velocity) is 75% less than in the nonpregnant state ($p < 0.005$) and remains unchanged during labor. This is true for both the high and the low affinity enzymes. These changes in the kinetic characteristics of the cyclic adenosine monophosphate–phosphodiesterase are indicative of noncompetitive inhibition. We conclude that this inhibition may be interpreted as part of the mechanism for uterine smooth muscle relaxation and pregnancy maintenance. (Am J Obstet Gynecol 1987;157:733-8.)

Key words: Human myometrium, phosphodiesterase activity, pregnancy

Pregnancy transforms the human uterus from a small, almost solid organ in the nonpregnant state to a large saccular organ with a volume of approximately 5 liters.[1] This dramatic change is characterized by relaxation along with hypertrophy and hyperplasia of the smooth muscle fibers of the uterine wall. Although the contribution of cyclic nucleotides to uterine growth in pregnancy has not been completely determined, increasing evidence suggests that cyclic adenosine monophosphate may play an important role in myometrial relaxation through the effects of this compound on intracellular ionized calcium concentration and myosin light-chain kinase activity.[2, 3] These actions include the following concepts: Cyclic adenosine monophosphate is thought to be the second messenger for β-adrenergic agonists that stimulate adenylate cyclase and induce relaxation. Increasing levels of cyclic adenosine monophosphate promote ionized calcium uptake by the sarcoplasmic reticulum, producing a decrease in cytosolic ionized calcium and thus promote relaxation. Finally, cyclic adenosine monophosphate–mediated phosphorylation of myosin light-chain kinase inhibits this enzyme's activity and promotes smooth muscle relaxation.[4]

Adenylate cyclase catalyzes production of cyclic adenosine monophosphate and phosphodiesterase catalyzes cAMP degradation. For a given production of cyclic adenosine monophosphate, the intracellular levels will depend on the activity of phosphodiesterase. Although phosphodiesterase activity in human myometrium at term has been measured,[5, 6] there have been no studies comparing phosphodiesterase activity from pregnant and nonpregnant human myometrium. This study was undertaken to investigate this matter and to compare the enzymatic activity between samples taken from women in active labor and samples from patients not in labor.

Material and methods

Subjects. Myometrium was obtained from the anterior wall of the uterine corpus of six nonpregnant women who underwent hysterectomy for benign gynecologic indications (adenomatous hyperplasia [two], uterine prolapse [one], endometriosis [two], and adenomyosis [one]). The specimen was obtained immediately after the uterine vessels were ligated. Myometrium was also obtained from uteri of six women not in labor who underwent elective repeat lower segment transverse cesarean section at term and from six women in labor who underwent primary lower segment transverse cesarean section for various obstetric indications (high breech in labor, active labor with maternal herpetic genital lesions, cephalopelvic disproportion, and fetal distress). All nonpregnant patients had an unremarkable medical history and did not receive any medications before hysterectomy. All six patients were operated on while under general anesthesia. All pregnant patients were healthy and their pregnancies were un-

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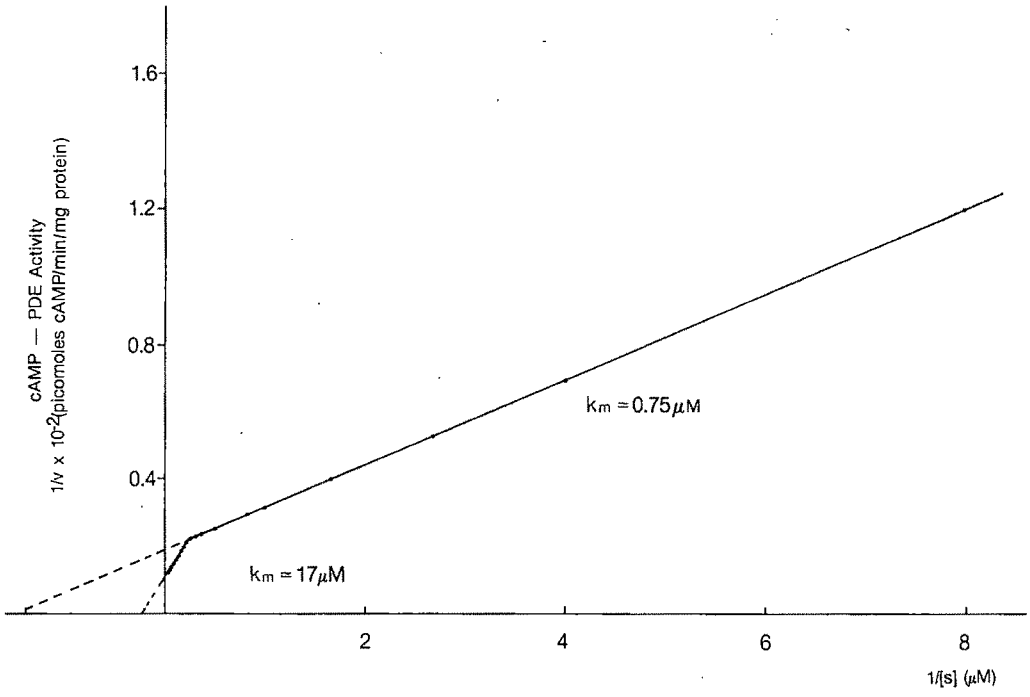


Fig. 1. Representative Lineweaver-Burk plot of human uterine cyclic adenosine monophosphate–phosphodiesterase activity versus substrate concentration. Evidence of two enzymatic forms with apparent Km values of approximately 0.75 and 17 μmol/L correspond to the high and low affinity enzyme, respectively (nonpregnant myometrium).

complicated until the time of delivery. None of the patients received uterotonic agents or other drugs. The procedure was performed with the patients under epidural anesthesia. The study was approved by the Clinical Research Practices Committee and each patient gave informed consent.

Tissue. The tissue biopsy specimen was taken from the upper edge of the transverse uterine incision from pregnant patients and from the lower part of the anterior uterine wall from nonpregnant patients. The tissue was rinsed in saline solution, blotted, and immediately frozen in a dry ice–acetone bath (−65° C). The entire procedure was performed within 60 seconds. Subsequently the tissue was stored at −70° C until assayed.

A 10% (wt/vol) homogenate in 0.25 mol/L sucrose, −10 mmol/L Tris hydrochloride (pH 8) was prepared (homogenization buffer). The frozen tissue was weighed in the frozen state, thawed in a small amount of buffer, and minced. The tissue was then homogenized at 4° C in a Polytron homogenizer for 30 seconds at three fourths of the maximum setting. The homogenate was then centrifuged for 60 minutes at 100,000 g and 4° C. Part of the supernatant was immediately assayed and the rest was stored at −70° C as a 1 : 1 solution in Tris hydrochloride–bovine serum albumin (0.5 mg bovine serum albumin per milliliter of 40 mmol/L Tris hydrochloride, pH 8). It was determined that freezing of the tissue and the homogenate did not change the enzyme activity for up to 6 months. The amount of protein was measured by a rapid and sensitive method that used the principle of protein-dye binding.[7]

Phosphodiesterase activity. Phosphodiesterase activity was measured in the supernatant by the two-step isotopic procedure as described previously by Thompson et al.[8] All assays, conducted in duplicate, were carried out in conditions of linearity with respect to time and protein concentration, allowing measurements of the initial rate of the reaction.

The assay mixture (0.4 ml) contained 5 mmol/L $MgCl_2$, 40 mmol/L Tris hydrochloride (pH 8), 4 mmol/L 2-mercaptoethanol, unlabeled cyclic adenosine monophosphate in different concentrations, labeled tritiated cyclic adenosine monophosphate (containing at least 100,000 cpm), and 0.1 ml of uterine cytosol. Reactions were initiated by the addition of enzyme; incubations were for 10 minutes at 30° C and were terminated by boiling for 45 seconds. After the addition of 0.1 ml of snake venom nucleotidase (1 mg/ml), the mixture was incubated for 10 minutes at 30° C and then placed in an ice bath. The entire content of the tubes was then transferred to Dowex 1-X8 (200 to 400 mesh) columns prepared in Pasteur pipettes (1 ml of 1 : 4 resin slurry was used to make each

column). The tritiated adenosine was then eluted with 2 ml of high-performance liquid chromatography grade methanol. The radioactivity of the eluate was determined in a liquid scintillation counter. In the range of substrate concentration from 0.125×10^{-6} to 100×10^{-6} mol/L, apparent Km (Michaelis constant) and maximum velocity values were calculated by Lineweaver-Burk plots by means of the computer program ENZPACK (Elsevier-BIOSOFT).

Statistical analysis. The differences in Km and maximum velocity values among the different groups were analyzed by one-way analysis of variance and Tukey's least significant difference test. A $\log_{10}$ transformation of the data was carried out to satisfy the prerequisite of homogeneity of variance when analysis of variance was used. A p value of ≤ 0.05 was considered significant.

Results

Kinetic properties of uterine cyclic adenosine monophosphate–phosphodiesterase. Kinetic analysis of cyclic adenosine monophosphate–phosphodiesterase activity versus cyclic adenosine monophosphate concentration by double reciprocal plots according to Lineweaver-Burk revealed nonlinear kinetic behavior suggestive of either multiple enzymatic forms or one form with negative cooperativity (Fig. 1). In nonpregnant myometrium in the range of cyclic adenosine monophosphate concentration from 0.125 to 4 μmol/L, an apparent Km value of 0.752 ± 0.046 μmol/L and maximum velocity of 2328 ± 197 pmol of cyclic adenosine monophosphate/min/mg of protein were obtained corresponding to the high affinity form of enzymatic activity. In the range from 5 to 100 μmol/L an apparent Km value of 17.68 ± 1.57 μmol/L and maximum velocity of 13,255 ± 1006 pmol/min/mg of protein were obtained corresponding to the low affinity form of enzymatic activity (Figs. 2 and 3).

In pregnant myometrium at term but not in labor the corresponding high affinity values were Km of 0.758 ± 0.038 μmol/L and maximum velocity of 604 ± 86 pmol/min/mg of protein. The corresponding low affinity values were Km of 22.31 ± 6.24 μmol/L and maximum velocity of 3570 ± 876 pmol/min/mg of protein (Figs. 2 and 3).

Similarly in pregnant myometrium in labor the high affinity values were Km of 0.674 ± 0.042 μmol/L and maximum velocity of 502 ± 100 pmol/min/mg of protein and the low affinity values, Km of 23.33 ± 4.44 μmol/L and maximum velocity of 2937 ± 464 pmol/min/mg of protein. The differences among the Km values were not significant but the differences in maximum velocity between nonpregnant and both pregnant groups were significant (analysis of variance, $p < 0.005$) (Figs. 2 and 3). These changes are suggestive of noncompetitive inhibition of uterine cyclic adenosine monophosphate–phosphodiesterase during pregnancy (Fig. 4).

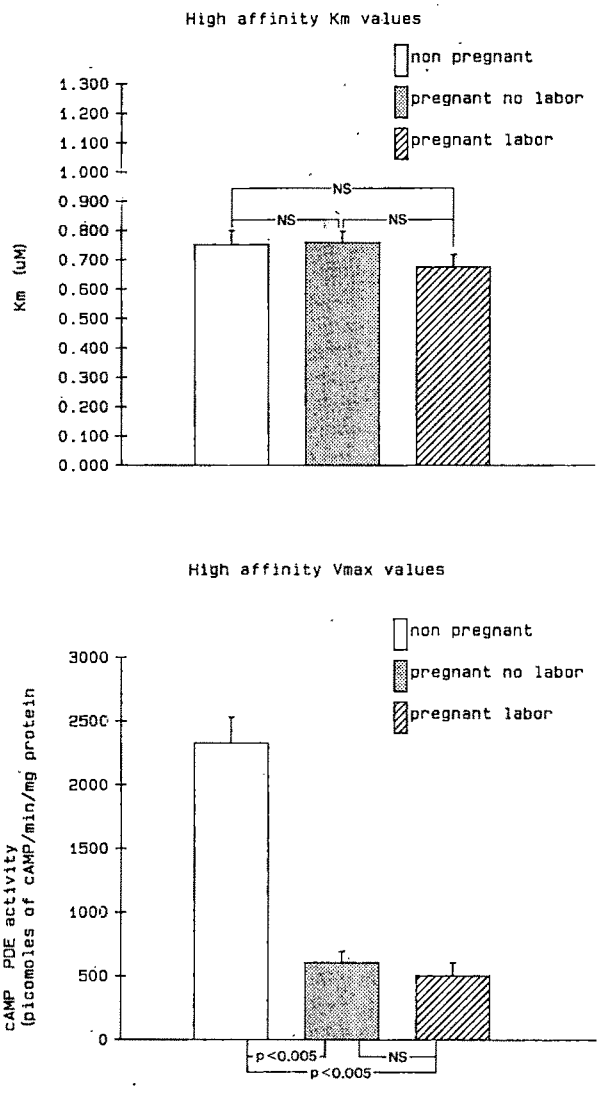


Fig. 2. *Top,* High affinity Km is similar in all three conditions. *Bottom,* High affinity, maximum velocity during pregnancy is approximately 75% less than in the nonpregnant state.

Comment

Normal pregnancy is a unique physiologic phenomenon that provokes major functional and anatomic changes in several organ systems. These changes create an environment necessary for the normal development of the conceptus and its maintenance in utero until maturity is accomplished. Cyclic nucleotides in general and especially cyclic adenosine monophosphate are generally thought to be involved in the control of the important structural and functional changes that occur



Fig. 3. *Top,* Low affinity Km is not significantly different in the three conditions. *Bottom,* Low affinity maximum velocity during pregnancy is also approximately 75% less than in the nonpregnant state.

in the uterus during the course of pregnancy.[2,3,6,9] Since the intracellular levels of cyclic adenosine monophosphate in part depend on the activity of cyclic adenosine monophosphate–phosphodiesterase, our findings suggest that in normal pregnancy a physiologic condition exists that decreases degradation of cyclic adenosine monophosphate, by inhibition of the activity of cyclic adenosine monophosphate–phosphodiesterase, and thus maintains adequate levels of this vital compound.

Although several investigators have examined cyclic adenosine monophosphate–phosphodiesterase activity in different species, few studies exist that have dealt with human myometrial cyclic adenosine monophosphate–phosphodiesterase.[5,10,11] Published studies of human uterine cyclic adenosine monophosphate–phosphodiesterase are contradictory concerning the kinetic characteristics of the enzyme. One group[5] reported the existence of two enzymes with apparent Km values of 12.6 μmol/L for the high affinity and 91 μmol/L for the low affinity enzyme and another group[11] reported the existence of one enzyme with an apparent Km of 1.56 μmol/L. This discrepancy may be related to differences in methodology, a frequent problem in cyclic adenosine monophosphate–phosphodiesterase research and one that makes comparisons difficult. Leroy et al.[6] reported the existence of two enzymatic forms with apparent Km values of 2.8 μmol/L for the high affinity enzyme and 57 μmol/L for the low affinity enzyme. These values are 2.5 times higher than values we report and this can be explained by the different substrate concentrations used. However, their findings are indicative of the existence of two enzymatic forms and are in agreement with our findings. In addition they resolved two peaks of phosphodiesterase activities on diethylaminoethyl cellulose chromatography. Peak I hydrolyzed both cyclic adenosine monophosphate and cyclic guanosine monophosphate and was activated by the ionized calcium–calmodulin complex and peak II was insensitive to ionized calcium–calmodulin and hydrolyzed only cyclic adenosine monophosphate.

Our findings support the existence of two enzymatic forms of cyclic adenosine monophosphate–phosphodiesterase in human myometrial cytosolic preparations and are also in agreement with animal studies in rats, rabbits, sheep, and primates.[2,12-16] The Km value of the high affinity enzyme in our studies remained fairly constant throughout, from the nonpregnant state to term pregnancy and subsequent labor. The low affinity Km value varied slightly but this change was not significant. However, the maximum velocity of both enzymes exhibited a 75% decrease from the nonpregnant to pregnant state. No significant difference exists in both the Km and maximum velocity values between samples from women in labor or not in labor. The lower activity of phosphodiesterase during pregnancy in comparison to the nonpregnant state may increase cyclic adenosine monophosphate levels and thus contribute to myometrial relaxation and pregnancy maintenance.

During active labor the activity of phosphodiesterase was similar to the activity of the enzyme obtained from women not in labor. The study design does not permit any conclusion regarding the significance of cyclic adenosine monophosphate–phosphodiesterase in the initiation of labor. Whether phosphodiesterase activity increases just before or during the early phase of labor and then returns to prelabor levels is a question that might best be examined in an animal model.

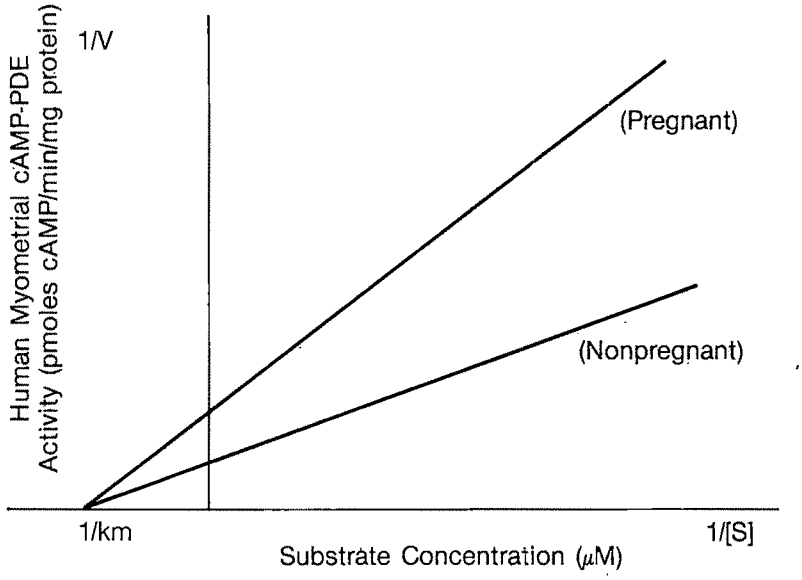


Fig. 4. Noncompetitive inhibition of human myometrial cyclic adenosine monophosphate–phosphodiesterase during pregnancy.

A potential criticism is the fact that the tissue was taken from the lower uterine segment, the least contractile part of the uterus. In one patient (not part of the present data) who underwent lower segment vertical cesarean section with extension to the upper segment, the enzyme activity between upper and lower segment was examined and was not different. In addition, all previous studies in humans examined tissue from the lower segment and this makes the comparisons more meaningful. Berg et al.[11] reported higher phosphodiesterase activity in women treated with terbutaline for preterm labor in comparison to a group of women who were not treated and were not in labor. They concluded that terbutaline was responsible for the increased phosphodiesterase activity, but a comparison between patients in and not in labor may render their conclusion invalid.

Consideration of myometrial phosphodiesterase has potential clinical applications. In the treatment of preterm labor β-agonists are frequently used. However, the use of these potent drugs has been associated with serious cardiopulmonary complications, which are dose related and may be fatal. Several selective phosphodiesterase inhibitors have been developed and are in clinical use as cardiotonic and antiarrhythmic agents.[17, 18] The development of similar selective inhibitors of myometrial phosphodiesterase might help us improve the efficacy of β-mimetic drugs and decrease the dosage required and thus the dose-related complications of these drugs. Conceivably the development of an inhibitor that is specific for myometrial cyclic adenosine monophosphate–phosphodiesterase could eliminate the serious cardiovascular complications associated with increasing levels of cyclic adenosine monophosphate in the myocardium.

In summary, human myometrial cytosol contains two enzymatic forms of cyclic adenosine monophosphate–phosphodiesterase, one low and one high affinity. The Km values, of both enzymes, are similar in the nonpregnant and term pregnant myometrium. However, the maximum velocity of phosphodiesterase in the term pregnant myometrium is 75% less than in the nonpregnant myometrium. Comparison of the enzyme from women in labor with the one from women not in labor did not reveal any differences in both Km and maximum velocity for both enzymatic forms. These findings suggest that cyclic adenosine monophosphate–phosphodiesterase may contribute to myometrial relaxation and pregnancy maintenance.

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Pregnancy outcome in medically complicated and uncomplicated patients aged 40 years or older

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Patients (183) who were delivered at age ≥40 years were studied to ascertain the nature and frequency of maternal and fetal complications at a single institution in a recent time period. These patients were further grouped into those of low parity, those who began pregnancy without underlying disease, and those who began pregnancy with underlying medical disorders. For the entire group preeclampsia, premature labor, precipitate labor, and malpresentation were significantly more common. The rate of vaginal delivery was substantially decreased, and serious postpartum morbidity was relatively common. The incidence of stillbirth, perinatal mortality, and abnormal birth weight was significantly increased. There were some differences in the nature and frequency of complications encountered among the subgroups, but no subgroup had a complication rate comparable to our general obstetric population. (Am J Obstet Gynecol 1987;157:738-42.)

Key words: Maternal age and pregnancy outcome, maternal and fetal complications

The effects of increasing maternal age on pregnancy outcome remain an unresolved issue for several reasons. Previous reports[1-5] have arrived at discordant conclusions, perhaps because advanced maternal age is associated with many other variables in patient characteristics. The older patients vary with respect to parity and the presence or absence of underlying medical disorders. This group is also socially diverse, with many women delaying childbearing because of professional or personal goals.[6] Conversely, there has been no decrease in the percentage of women delivering at age ≥40 years in our population of medically and economically indigent patients. The present study is an attempt

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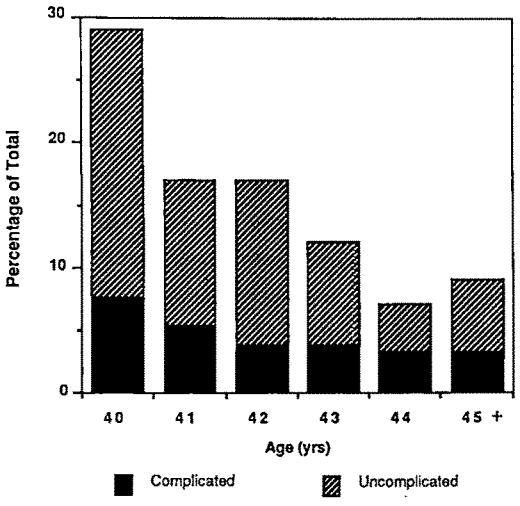


Fig. 1. Bar graph of age distribution within the study group. Results are given as percentage of entire study group. Patients with medical complications *(solid bars)* are compared with patients without medical complications *(hatched bars)*.

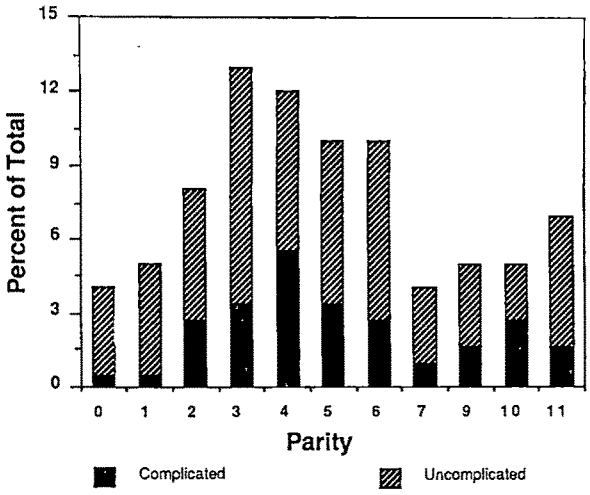


Fig. 2. Bar graph of parity distribution within the study group. Results are given as percentage of entire study group. Patients with medical complications *(solid bars)* are compared with patients without medical complications *(hatched bars)*.

to ascertain the extent of complications encountered in a recent time period. We hypothesized that any increase in complications could be attributed to the presence of medical disorders that antedated the pregnancy or to high parity rather than to advanced maternal age per se.

Material and methods

Delivery logbooks of Charity Hospital at New Orleans from May 1, 1983 through April 30, 1986 were used to identify women who were aged ≥40 years with a gestation of >20 weeks at the time of delivery. The maternal charts were retrieved from medical records; no patient whose chart was complete was excluded for any reason. Of the 196 charts, 183 were obtained and individually reviewed, the data were abstracted onto a predesigned form, any readmission within 42 days was also reviewed to ascertain if a delayed complication had occurred. The patients studied were divided into subgroups on the basis of the presence or absence of medical disorders before the pregnancy; a subgroup of patients of low parity was formed consisting of all patients who were para 2 or fewer at the time of admission for the index delivery. Results were compared with the outcomes recorded in our computerized data base for all patients on the Tulane obstetric service at Charity Hospital. Differences were subjected to statistical analysis with a binomial independent test with significance accepted if $p < 0.05$.

Results

During this time interval there were 9769 deliveries on the Tulane service; the 183 patients aged ≥40 years constituted 1.87% of our obstetric population. The age distribution within the study group was skewed toward the lower range; only 16 of the patients were aged ≥45 years. The average age for the entire study group was 41 years 7 months. Of these patients, 150 were para 3 or more at the time of admission for delivery of the index pregnancy. The distributions of age and parity are given in Figs. 1 and 2. The obstetric complications (Table I) of pregnancy-induced hypertension and abruptio placentae, premature labor, precipitate labor, and malpresentation were all increased. There were only two cases of placenta previa, which is not different from the 0.4% incidence seen in our general obstetric population. The percentage of women who were delivered vaginally (74.3%) was decreased, but the rate of delivery by repeated cesarean section (6.5%) was not increased. Stillbirth and perinatal mortality were abnormally frequent. Of the birth weights, 75% were normal, with 17.5% of the infants weighing <2500 gm and 7.6% of the infants weighing >4000 gm. In comparison, 86% of our entire population of infants weigh between 2500 and 4000 gm at birth. Hemorrhage that required transfusion was seen in 6% of the patients, and there was a trend toward increased febrile morbidity. Deep venous thrombosis, thrombotic pulmonary emboli, or pulmonary edema was seen post partum in eight patients, and seven patients required admission to the intensive care unit.

Fifty patients with an average age of 42 years 3 months had medical disorders that antedated the pregnancy. Of these 50 patients, 37 had hypertension, 15 had diabetes, and 10 had both hypertension and diabetes. The remaining medical disorders included

Table I. Obstetric complications in the study group and reference hospital population (results are given as percentages)

Result	*Medically complicated n = 50*	*Medically uncomplicated n = 133*	*Low parity n = 33*	*Entire group N = 183*	*Reference population N = 9769*
Preeclampsia	24*	12.7	24*	15.8*	10
Eclampsia	4	0.7	—	1.6	0.3
Abruptio placentae	2	3.7	3	3.2*	0.4
Premature labor	24*	15.7*	9	18*	9
Precipitate labor	12	18.7*	3	16.9*	4
Malpresentation	14*	8.2*	12*	9.8*	3.5
Fetal distress	10*	2.2	15*	4.3	3

*p = <0.05.

Table II. Route of delivery in the study groups and reference hospital population (results are given as percentages)

Route of delivery	*Medically complicated n = 50*	*Medically uncomplicated n = 133*	*Low parity n = 33*	*Entire group N = 183*	*Reference population N = 9769*
Vaginal delivery	58*	80.4	51*	74.3*	81
Primary cesarean	32*	10.5	40*	16.4	14
Repeated cesarean	6	6.7	6	6.5	5
Cesarean hysterectomy	4	2.2	3	2.7	0.4

*p = <0.05.

neuropsychiatric disease, arteriosclerotic heart disease, asthma, tuberculosis, collagen vascular disease, chronic hepatitis, and syphilis. Seven of these patients had more than one underlying problem. The maternal and fetal outcomes for this subgroup are worse than the general population; antepartum complications (Table I) of preeclampsia and premature labor are both elevated as are fetal complications of malpresentation and fetal distress. These women were delivered more often by cesarean section (Table II); 43% of the cesarean sections were done for fetal distress (diagnosed by severe variable or persistent late decelerations), 25% for malpresentation, and 25% for failure to enter labor or obtain normal progress during obstetrically indicated induction of labor. Pulmonary edema, deep venous thrombosis, and pulmonary emboli were seen as postpartum complications (Table III) in 14% of the subgroup with medical disorders and were the cause of admission to the intensive care unit for 10% of this subgroup. Two cases of pulmonary emboli occurred during the second to sixth weeks post partum, which required readmission to the hospital and intensive care.

Patients (133) with an average age of 41 years 7 months did not have underlying medical disorders at the inception of pregnancy. Of these women, 110 were para 3 or more at the time of admission for the index pregnancy; this distribution of parity is equivalent to that of the entire study group. These women experienced less preeclampsia and fetal distress, but premature labor remained significantly elevated, and precipitate labor appeared to be a substantial problem. The route of delivery for these patients is not different from that for the entire obstetric population. The stillbirth rate remains elevated. A birth weight of <2500 gm is not different from the general population, but a birth weight of >4000 gm is more common (Table IV). Twelve percent were noted to have uterine atony or required manual removal of a retained placenta; two thirds of these patients also required a transfusion, which resulted in an elevated rate of transfusion for this subgroup.

Thirty-three patients with an average age of 41 years 1 month were para 2 or less on admission for the index delivery. Of these patients, 70% did not have underlying medical disorders; this distribution is equivalent to that of the entire group. These lower parity patients also had an increased incidence of preeclampsia, malpresentation, and fetal distress. This subgroup had the highest rate (49%) of cesarean sections. Failure to progress accounted for half of the cesarean sections, and the remainder were evenly divided between malpresentation and fetal distress. This group also has a high stillbirth and perinatal mortality rate, but the stillbirths occurred in women with serious underlying medical disorders. There is again a tendency toward an abnormal birth weight, with only 70% of infants weighing between 2500 and 4000 gm. The number of infants who weighed <2500 or >4000 gm in this sample is too

Table III. Postpartum complications in the study groups and reference hospital population (results are given as percentages)

Result	*Medically complicated n = 50*	*Medically uncomplicated n = 133*	*Low parity n = 33*	*Entire group N = 183*	*Reference population N = 9769*
Febrile morbidity	10	12.7	15	12	7
Hemorrhage and transfusion	2	7.5*	9*	6*	2
Deep venous thrombosis	4	0.7	3	1.6	NA
Pulmonary emboli	4	—	—	1	NA
Pulmonary edema	6	—	3	1.6	NA

NA = Data not available.
*p = <0.05.

Table IV. Perinatal data from the study groups and reference hospital population (results are given as percentages)

Result	*Medically complicated n = 50*	*Medically uncomplicated n = 133*	*Low parity n = 33*	*Entire group N = 183*	*Reference population N = 9769*
Stillbirth	6	3*	6	3.8*	0.9
Perinatal mortality	12*	3.7*	5	6*	2
Congenital anomalies	2	0.7	—	1	0.5
Birth weight <2500 gm	32*	12	24	17.5*	10.5
Birth weight 4000-4499 gm	8	4.5*	6	5.4*	2.5
Birth weight ≥4500 gm	6	0.7	—	2.2	0.8

*p = <0.05.

small to reach statistical significance as separate groups. Peripartum hemorrhage that required transfusion occurred in 9% of the patients of lower parity. This is elevated with respect to the general obstetric population and was attributable to placenta previa, abruptio placentae, and one case of uterine atony at cesarean section after a prolonged second stage. The two patients with deep venous thrombosis or pulmonary edema had underlying medical disorders.

Comment

Other studies[7-9] have also noted increases in the complication rates for both mother and baby when maternal age increases. Social position, parity, and the presence or absence of underlying medical disorders must also be considered to affect obstetric outcome. In our study there was no correlation between low or high parity and the presence or absence of preexisting disease. This implies that any difference in outcome between the three subgroups is not attributable to confounding the effects of parity with the general medical health of the patient.

In patients without underlying medical disorders the absence of chronic hypertension appeared to protect them from high rates of preeclampsia. However, their general good health did not protect them from increased rates of other maternal and fetal complications, including stillbirth. These patients delivered heavier infants. Of these patients, 16% were estimated to be <36 weeks when delivered, but only 12% of the infants weighed <2500 gm. The older women were more likely to have hypertension, diabetes, or both than younger patients. As would be expected, the incidence of preeclampsia was elevated in those patients of low parity or with underlying hypertension and was associated with increased rates of prematurity, low birth weight, malpresentation, and fetal distress. The patients of lower parity had high rates of preeclampsia but normal rates of prematurity. They tended to develop preeclampsia that was later in onset and of milder severity than the women with underlying medical disease. Because the onset of disease tended to be later, their rate of prematurity was not as high. The highest cesarean section rate is seen in the subgroup of lower parity, presumably because these women did not have the advantage of previous vaginal deliveries. The 23 women of low parity who had no underlying medical diseases encountered complication rates that appeared similar to the entire group. However, the small numbers preclude statistical certainty that their outcome is comparable. Small statistical advantages to being of low parity and in good general health would be missed with this sample size.

The incidence of premature delivery is elevated in patients with and without medical complications but not in the subgroup of lower parity. Thus the overall ele-

vated prematurity rate appears to be associated with either the presence of underlying medical disorders or with high parity. Other studies[7] have not consistently found an increase in the prematurity rates of older, healthy parous women. Malpresentation is increased in all the subgroups and is not simply a reflection of prematurity or high parity, since it is increased in the subgroup of women of low parity, who do not have an increased prematurity rate. Fetal distress is elevated in the medically complicated subgroup, and the five patients in the low-parity group with the diagnosis of fetal distress all had underlying medical disorders. The observation that birth weight is increased in the group without medical complications, together with the absence of fetal distress, implies that advanced maternal age alone does not impair placental function or fetal growth. The increased rates of stillbirth and perinatal mortality in the medically uncomplicated group are attributable primarily to prematurity but also to abruptio placentae.

As has been noted in other studies,[7, 10, 11] the cesarean section rate is increased in these patients. When patients of low parity with medical complications are excluded, the vaginal delivery rate is equivalent to the general obstetric population. However, 60% of our general obstetric population are primiparas or nulliparas, and their cesarean section rate should be higher than a group of women with this parity distribution. The rate of repeated cesarean sections is not elevated and cannot account for the overall increase in cesarean sections. In our hospital fetal distress and malpresentation are the indication for about 20% of the primary cesarean sections. These two diagnoses were the indication for about 50% of the primary cesarean sections performed in the study group and account for some of the increase in the cesarean section rate. The other major indication for cesarean section was failure to progress in labor. This problem may be attributable to cephalopelvic disproportion associated with increased birth weight or to our inability to always successfully induce normal labor whenever delivery is deemed appropriate for maternal or fetal indications.

We found four patients in the group with medical complications who had deep venous thrombosis, two of whom also had pulmonary emboli. This is equivalent to an incidence of 1:12 for thrombosis and 1:25 for pulmonary emboli. The usual rates for these disorders quoted in the obstetric literature[12] are 1:100 for postpartum deep venous thrombosis and 1:750 for pulmonary emboli. Pulmonary edema and admission to the intensive care unit also appear to be considerably more frequent than usual. Rates for these complications do not appear in the general literature, and we do not have them for our hospital. Although there was no maternal mortality in these 183 patients, serious maternal morbidity was observed. Preeclampsia, complications of cesarean section, obstetric hemorrhage, pulmonary edema, and pulmonary emboli are all frequently quoted[13, 14] as causes of maternal mortality, and we would expect a larger study to validate the elevated maternal mortality associated with advanced maternal age.

In conclusion, we believe that patients considering delivery at age ≥40 years should be advised that there is an increased risk of both maternal and fetal complications. The outcome is more likely to be unfavorable at age ≥40 years, even when the patient is of low parity or in good general health. Those patients of lower parity should expect no reduction in their overall complication rate and can be expected to have a higher rate of cesarean section. There is a further increase in the complication rate for patients with underlying medical disorders; most of this increase is probably attributable to an increased rate of preeclampsia that tends to be severe and relatively early in onset. It seems possible that deliberate alterations in the pattern and content of obstetric care, which take into account these specific complications could have a favorable impact on the maternal and fetal outcome.

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Patterns of human fetal heart rate accelerations from 26 weeks to term

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Computerized analysis of the distribution of 2598 fetal heart rate accelerations in 83 healthy fetuses at 26 to 40 weeks' gestation demonstrated that the currently used definition of an acceleration as ≥15 beats/min for ≥15 seconds is applicable only after 30 weeks' gestational age in fetuses with a basal fetal heart rate of ≤128 beats/min. A significant negative correlation was found between the mean hourly basal fetal heart rate and the mean amplitude of fetal heart rate accelerations from 30 weeks to term. There was also a significant maturational process in the pattern of fetal heart rate and fetal heart rate accelerations that occurred between 26 and 28 and between 30 and 32 weeks; this was characterized by a decrease in basal fetal heart rate, an increase in the amplitude of fetal heart rate accelerations, and an increase in long-term fetal heart rate variability. (AM J OBSTET GYNECOL 1987;157:743-8.)

Key words: Nonstress test; fetal heart rate accelerations during third trimester; effect of gestational age on nonstress test

Antepartum fetal heart rate (FHR) testing, including nonstress and contraction stress testing, has been seriously criticized[1, 2] because of a low positive predictive value, absence of strict and uniform standards for interpretation, and the potential effect of gestational age on FHR reactivity. In addition, until recently accurate recording and interpretation of human FHR with Doppler ultrasound have presented major problems because of a signal loss approaching 60% at 26 to 28 weeks' gestational age.[3] However, signal loss has been markedly reduced by newer autocorrelation-based FHR monitors.[4] In order to provide a better understanding of antepartum FHR testing, the aims of this study were to analyze the distribution of FHR accelerations from 26 weeks' gestational age to term and to examine the influence of the basal FHR on the amplitude of FHR accelerations.

Patients and methods

Patients. Informed consent was obtained from 89 healthy women with pregnancies between 26 and 40 weeks' gestational age. Eighty-three women and fetuses remained in good health, and six were excluded from the study. Five women were delivered of their infants before 37 weeks: Two had abruptio placentae, two had premature labor, and one was delivered of a stillborn infant at 35 weeks (4 weeks after study): All complications were of unknown cause. Another patient was lost to follow-up. The remaining 83 women had normal deliveries at term that confirmed gestational age and the good health of fetuses studied. The average gestational age at delivery was 39.3 ± 0.1 weeks (SEM). The average weight of fetuses was 3350 ± 42 gm. The average 5-minute Apgar score was 9.3 ± 0.1 (mode 9; range 7 to 10). No Apgar score was less than 7 at 5 minutes. Umbilical arterial cord blood samples were obtained at birth in 58 of the 83 fetuses. The mean umbilical arterial P_{O_2} was 17.8 ± 1.0 mm Hg, P_{CO_2} was 50.0 ± 1.3 mm Hg, and pH was 7.27 ± 0.01. Ten (13.7%) women were delivered of their infants by cesarean section: Two developed acute fetal distress in labor (5-minute Apgar scores of 8 and 10, respectively), one had cephalopelvic disproportion, one had a transverse lie at the onset of labor, and six had repeat cesarean sections. Eighty-one fetuses were in vertex presentation and two were breech at the time of delivery.

Experimental design. The women received a standardized 800 kcal breakfast or lunch at 0800 or 1200 hours. All studies began 1 hour later and were conducted in a quiet room with patients either sitting upright in bed or resting in a lateral recumbent position. Women were asked not to smoke for at least 4 hours before studies. Smoking or food intake by patients or observers was not permitted during the study. Women were studied continuously for 1 hour. Nine women participated in more than one study, and these were

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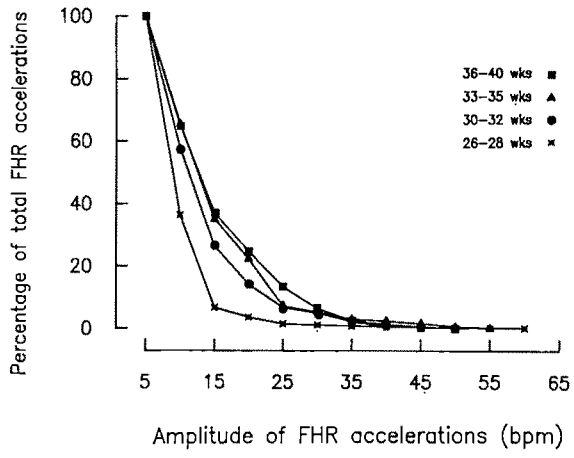


Fig. 1. A cumulative histogram of 2598 FHR accelerations of ≥15 seconds' duration was plotted in successive increments of 5 beats/min to define the distribution of acceleration amplitudes in fetuses from 26 to 40 weeks' gestational age.

conducted in different gestational age groups. Twenty-three studies were done at 26 to 28 weeks, 24 at 30 to 32 weeks, 26 at 33 to 35 weeks, and 28 at 36 to 40 weeks, for a total observation time of 101 hours.

Fetal heart rate measurements. Fetal heart rate was recorded with a Hewlett-Packard 8040A (Hewlett-Packard GMBH, Boeblinger, Federal Republic of Germany) external monitor by use of a compact transducer and was analyzed on-line by a Sage II microprocessor (Sage Computer Technology, Reno, Nevada) as previously described.[5] The FHR intervals were examined by means of an artifact detection algorithm,[6] and valid pulse intervals were averaged over 3.75 seconds. During on-line analysis a baseline was fitted to the heart rate record,[7] and accelerations of ≥10 beats/min for ≥15 seconds above the baseline were recognized automatically.

For each 10-minute interval the basal FHR (beats per minute), the mean minute range (milliseconds),[4] and the number of FHR accelerations were calculated. The mean minute range value was calculated as the difference between the longest and the shortest RR intervals every minute and averaged for the length of observation. The mean minute range is a computer-derived index of long-term FHR variability.[4]

Fetal breathing movements and gross fetal body movements were measured with a real-time ADR ultrasound scanner (Model 2130; Advanced Diagnostic Research Corp., Tempe, Arizona) and will be reported elsewhere.

Data analysis. The basal FHR, the number of FHR accelerations of ≥10 beats/min for ≥15 seconds above the baseline, and amplitude of individual FHR accelerations were determined on line in successive 10-minute intervals. The number of FHR accelerations of ≥15 seconds' duration were also automatically determined off-line for acceleration amplitudes of 5 beats/min and in successive increments of 5 beats/min to a maximum amplitude of 60 beats/min.

Portions of records that could not be analyzed owing to FHR signal loss of more than 30% or patient interruption were not included and made up 5.2% of total study time. The average signal loss of the remaining 96 hours included in this analysis was 6.9% ± 0.9%, 5.3% ± 0.6%, 5.7% ± 0.8%, and 4.5% ± 0.5% at 26 to 28, 30 to 32, 33 to 35, and 36 to 40 weeks', respectively.

Results are presented as grouped means and standard errors of the mean. The significance of differences in the means was examined by Bonferroni t test statistic tables to allow for multiple comparisons.[8] Standard regression analyses with the Pearson product-moment correlations (r) were determined. Regression lines were compared for co-incidence by means of regression procedures described by Kleinbaum and Kupper.[9] This analysis was performed with the package MINITAB (MINITAB Data Analysis Software, State College, Pennsylvania).

Results

Distribution of FHR accelerations. Fig. 1 is a cumulative histogram plot of 2598 FHR accelerations of ≥15 seconds above the baseline in 83 fetuses between 26 and 40 weeks' gestational age. The percentage of total FHR accelerations was calculated in successive increments of 5 beats/min to define the distribution of acceleration amplitudes. Of FHR accelerations between 30 and 40 weeks' gestation, 31.7% were ≥15 beats/min for ≥15 seconds. However, only 6.7% of FHR accelerations were ≥15 beats/min for ≥15 seconds at 26 to 28 weeks.

The percentage of time intervals during which fewer than two FHR accelerations of ≥15 beats/min for ≥15 seconds occurred is plotted in 10-minute increments (Fig. 2, *A*). From 30 to 40 weeks, 57.5% of tracings had fewer than two FHR accelerations at 10 minutes and 2.7% at 60 minutes. However, at 26 to 28 weeks, 95.3% of recordings had fewer than two FHR accelerations at 10 minutes, 67.0% at 40 minutes, and 65.0% at 60 minutes, suggesting that most of the FHR accelerations in this group were <15 beats/min and cannot be detected with an increase in the observation time. However, using a minimum amplitude of 10 beats/min for ≥15 seconds to define an acceleration at 26 to 28 weeks, the length of time from the beginning of recordings until two FHR accelerations occurred was similar to that at 30 to 40 weeks (Fig. 2, *A*).

In Fig. 2, *B*, it can be seen that with a decrease in the minimum amplitude to define an acceleration, there was a concomitant decrease in the percentage of 10-minute intervals during which fewer than two FHR accelerations occurred. This was observed at all gestations from 26 to 40 weeks.

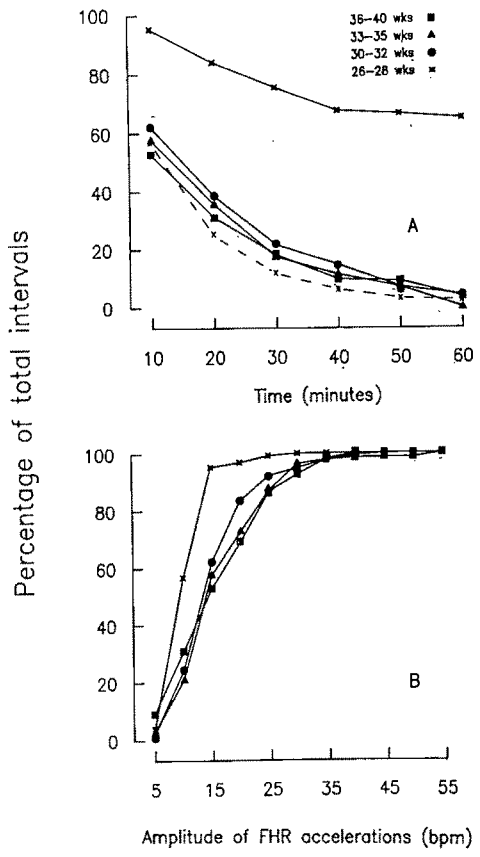


Fig. 2. A, The percentage of time intervals during which fewer than two FHR accelerations of ≥15 beats/min for ≥15 seconds occurred was plotted for all fetuses from 26 weeks to term. The percentage of time intervals during which fewer than two FHR accelerations of ≥10 beats/min for ≥15 seconds was also plotted for fetuses from 26 to 28 weeks on the same time scale (- - - -). **B,** Cumulative percentage of 10-minute intervals during which fewer than two FHR accelerations occurred was plotted in successive increments of 5 beats/min to define the minimum amplitude for an acceleration in all fetuses from 26 to 40 weeks.

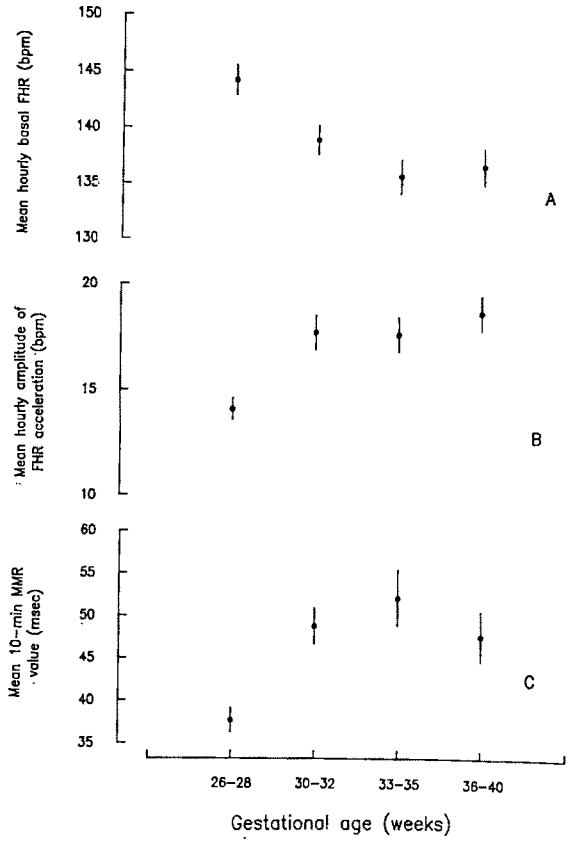


Fig. 3. A, The mean hourly basal FHR (±SEM) was plotted for fetuses at 26 to 28, 30 to 32, 33 to 35, and 36 to 40 weeks' gestation. **B,** The mean hourly amplitude of FHR accelerations and, **C,** the mean 10-minute mean minute range *(MMR)* were also plotted on the same scale.

Relationship between gestational age, basal FHR, and amplitude of FHR accelerations. Mean hourly basal FHR was 144.4 ± 1.3 beats/min at 26 to 28 weeks, which was significantly higher than at 30 to 32, 33 to 35, and 36 to 40 weeks ($p < 0.05$, $p < 0.01$, $p < 0.01$, respectively) (Fig. 3, *A*). Figure 3, *B*, demonstrated that there was a significant increase in the mean amplitude of FHR accelerations from 14.1 ± 0.5 beats/min at 26 to 28 weeks to 17.7 ± 0.8 beats/min at 30 to 32 weeks ($p < 0.01$), and this remained higher at 33 to 35 and 36 to 40 weeks (both $p < 0.01$) (Fig. 3, *B*). Finally, the 10-minute mean minute range was lower at 26 to 28 weeks than at 30 to 32, 33 to 35, and 36 to 40 weeks (all $p < 0.01$) (Fig. 3, *C*). It was important that there was no difference in the mean FHR, acceleration amplitude, and mean minute range between 30 and 40 weeks' gestational age.

There was a significant negative correlation between the mean hourly amplitude of FHR accelerations of ≥10 beats/min for ≥15 seconds and the mean hourly basal FHR at 30 to 32, 33 to 35, and 36 to 40 weeks' gestation (Fig. 4, *B* to *D*), which demonstrated that within individual fetuses at 30 to 40 weeks the higher the mean hourly heart rate, the lower the height of individual FHR acceleration. No such correlation was found at 26 to 28 weeks (Fig. 4, *A*).

A comparison of the regression lines of the relationship of FHR acceleration amplitudes and basal FHR at 30 to 40 weeks, by means of regression procedures described by Kleinbaum and Kupper,[9] confirmed that the regressions of Fig. 4, *B* to *D*, were coincident (slopes and intercepts were equal). Therefore these three sets of data were pooled, and Fig. 5 demonstrated the regression of FHR acceleration amplitude with basal FHR for fetuses at 30 to 40 weeks' gestation. The 95% confidence interval is indicated. It can be seen that a minimum acceleration amplitude of ≥15 beats/min for ≥15 seconds will detect more than 97.5% of FHR accelerations only if the basal FHR is ≤128 beats/min. However, defining the minimum amplitude of FHR as ≥10 beats/min for ≥15 seconds will detect more than

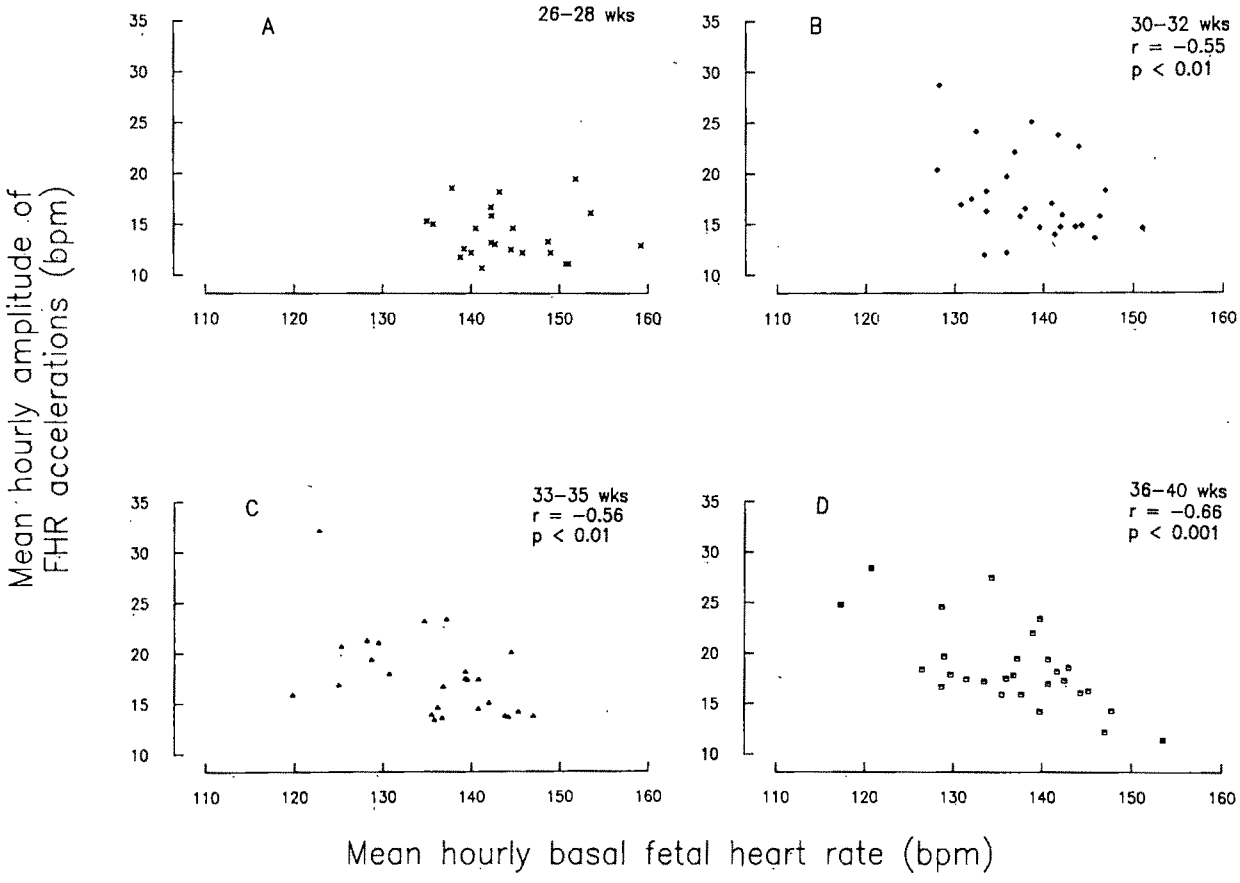


Fig. 4. There was a significant negative correlation between the mean hourly amplitude of FHR accelerations and the mean hourly basal heart rate at all gestations from 30 to 40 weeks **(B, C, D)**. No correlation was found at 26 to 28 weeks' gestation **(A)**.

97.5% of FHR accelerations with a basal FHR of ≤143 beats/min in fetuses of 30 to 40 weeks' gestational age.

Comment

Analysis of the distribution of FHR accelerations in healthy fetuses from 26 to 40 weeks' gestation demonstrated that the currently used definition of an acceleration as ≥15 beats/min for ≥15 seconds is applicable only after 30 weeks' gestational age. Decreasing the minimum amplitude to define an acceleration to 10 beats/min will have two effects on nonstress testing: (1) The length of time necessary to have a reactive nonstress test at 26 to 28 weeks will follow the same pattern as that at 30 to 40 weeks with 15 beats/min as a definition and, (2) there will be an improvement in recognition of FHR accelerations at a basal heart rate of >128 beats/min after 30 weeks' gestation.

The data also demonstrated a maturational process in the pattern of FHR and FHR accelerations that occurred between 28 and 30 weeks and was characterized by: (1) a decrease in basal FHR of 5 beats/min, (2) an increase in the amplitude of FHR accelerations of 4 beats/min, (3) an increase in long-term FHR variability, and (4) the appearance of a significant negative correlation between the mean amplitude of FHR accelerations and the mean basal FHR. It was interesting that no further significant changes occurred in these measurements between 30 weeks and term.

Current literature, between 1974 and 1984, evaluating the predictive value for fetal outcome with the nonstress test has been reviewed by Devoe et al.[1] They reported an average sensitivity of 48%, specificity of 93%, positive predictive value of 38%, and negative predictive value of 95%. A major problem in the methodology of the studies reviewed was the criteria used for test interpretation. There were 21 different definitions for a "reactive" nonstress test in the 44 studies. In addition, the gestational age of fetuses undergoing testing was frequently omitted.[2]

Brown and Patrick[10] increased the length of FHR recording up to 120 minutes using the rationale that two episodes of gross fetal body movements would normally have been predicted during that time.[11] The increase in observation time did not alter the negative predictive value of a reactive test defined as five or more

FHR accelerations of ≥15 beats/min for ≥15 seconds during any 20-minute interval. The present data from healthy fetuses indicated that this is an acceptable strategy for nonstress testing of fetuses from 30 to 40 weeks' gestation, because at 60 minutes only 2.7% of fetuses had fewer than two accelerations of ≥15 beats/min for ≥15 seconds. In contrast, 65.0% of the fetuses at 26 to 28 weeks in this study had fewer than two FHR accelerations of ≥15 beats/min for ≥15 seconds at 60 minutes (Fig. 2, *A*). However, with a definition of ≥10 beats/min for ≥15 seconds for an acceleration in fetuses of 26 to 28 weeks' gestation, only 4.0% had fewer than two FHR accelerations at 60 minutes (Fig. 2, *A*).

Fig. 1 indicated that in fetuses of 26 to 28 weeks' gestation 35.0% of FHR accelerations were ≥10 beats/min for ≥15 seconds, which was similar to 31.7% of FHR accelerations that were ≥15 beats/min for ≥15 seconds at 30 to 40 weeks' gestation. Therefore recognition of FHR accelerations before 30 weeks is only feasible when a minimum amplitude of ≥10 beats/min for ≥15 seconds is used to define an acceleration.

Dawes et al., using an improved method of fitting a baseline to normal human FHR traces,[7] demonstrated a large rise in the numbers of accelerations after 28 weeks.[12] They also reported a significant negative correlation between FHR variation, as expressed as the interquartile range of pulse intervals, and basal heart rate.[12] However, because of the larger scatter in the results the authors suggested that there was no material advantage to be gained by expressing the variability in FHR in terms of the basal FHR.

A previous study of healthy fetuses near term showed that the mean hourly FHR was increased by 18 beats/min over 24-hour periods during times of gross fetal body movements.[13] Further, the mean daily amplitude of FHR was inversely related to the mean daily FHR.[14] Our data confirmed these findings. We also demonstrated that this same relationship was present from 30 weeks to term. This might indicate that individual fetuses after 30 weeks' gestation have a set "gain" for FHR accelerations that is related to the basal heart rate.

Dalton et al.[15] demonstrated in fetal lambs that simultaneous parasympathetic and β-sympathetic blockade reduced heart rate to a greater extent in the younger fetuses. They suggested that there might be a resting cardioacceleratory drive that diminishes with age in fetal lambs. Whether or not this was related to a reduction in catecholamine sensitivity with increasing β-sympathetic dominance during gestation remains speculative. However, this hypothesis could explain the decrease in basal FHR and the increase in long-term FHR variability reported between 26 to 28 and 30 to 32 weeks in this study. It remains to be determined if this reflected a functional maturation of the fetal autonomic nervous system, particularly the predominance of the β-sympathetic system.

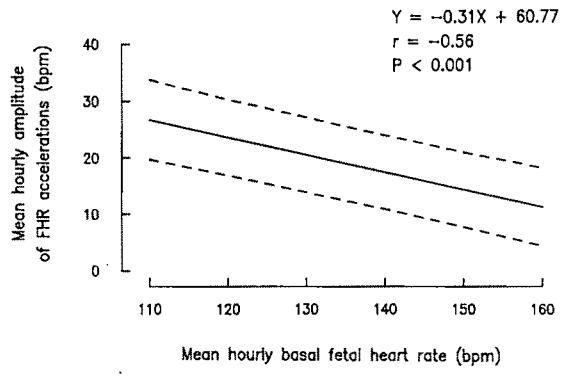


Fig. 5. Regression line (——) of the mean hourly amplitude of FHR accelerations was plotted in relationship to the mean hourly basal heart rate in all fetuses from 30 to 40 weeks with the 95% confidence interval (- - - -). A definition of ≥15 beats/min for ≥15 seconds will detect more than 97.5% of all FHR accelerations only if the basal FHR is ≤128 beats/min.

It is important that previous reports[4, 12, 16] and the current data suggest that the changes in basal FHR, amplitude of FHR accelerations, and long-term FHR variability occur during a critical and narrow period between 28 and 30 weeks' gestational age. Furthermore, no additional significant changes were measured between 30 and 40 weeks' gestational age. This suggests that the autonomic control of FHR in healthy human fetuses might be fully mature by 30 weeks' gestation. Data are not yet available in fetuses of less than 26 weeks' gestation.

Using computerized analysis of FHR we have demonstrated in a group of highly selected healthy fetuses that currently used criteria for nonstress testing are significantly affected by the basal FHR and gestational age. We have used instrumentation that improved heart rate recording but is not available yet for many persons. Twenty-three patients were included in the 26- to 28-week group, which represented a relatively small sample size, but the data suggested that using an amplitude of 10 beats/min to define an acceleration could be a strategy to increase the chance of detecting FHR accelerations before 30 weeks' gestational age. However, this hypothesis should be clinically tested before a new definition for an acceleration is advocated. During the last 10 years there has been a dramatic decrease in neonatal deaths and morbidity in premature newborns of 26 to 28 weeks' gestational age, without any major improvements in use of FHR evaluation of fetal health at this age. Future studies evaluating antepartum FHR testing should also consider the value of adding other FHR information such as baseline heart rate, heart rate variability, and gestational age in order to increase accuracy to predict fetal outcome with nonstress testing.

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Riboflavin concentration in maternal and cord blood in human pregnancy

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Riboflavin concentration was measured in sera of a control population and in a series of paired maternal and cord sera. The assay technique was carefully validated and appears to be specific and reproducible. The mean riboflavin concentration in 12 apparently healthy adults was 116 ± 46 nmol/L (SD). In 20 uneventful pregnancies the cord serum concentration was generally higher than the maternal concentration (158 ± 47 nmol/L versus 113 ± 35 nmol/L; p = 0.001). The cord-to-maternal ratio in paired sera averaged 1.45 ± 0.44. There was no detectable difference in binding of riboflavin to cord and maternal serum proteins as measured by equilibrium dialysis (59.0% ± 17% versus 60.8% ± 16%). Comparison of protein binding by paired cord and maternal sera yielded a ratio of 0.99 ± 0.13. The transplacental gradient of riboflavin concentration is unrelated to protein binding and is consistent with active transport by the placenta, as previously demonstrated in vitro. (AM J OBSTET GYNECOL 1987;157:748-52.)

Key words: Riboflavin concentration, maternal, cord, protein binding

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Riboflavin is an essential nutrient that must be delivered to the fetus across the placenta. The human placenta, in vitro, actively transfers riboflavin from the maternal to the fetal circulation, establishing a gradient toward the fetus.[1,2] The situation in vivo is less certain.

Table I. Riboflavin concentration in maternal and cord blood at delivery, as reported in the literature

Reference	*Sample*	*n*	*Maternal (nmol/L)*	*Cord (nmol/L)*	*Gradient (C/M)**
Baker et al.[9]	Blood	14	675 ± 224 (SD)	896 ± 238	1.3
Lust et al.[10]	Serum	10	15.4 ± 0.33 (SEM)	59.9 ± 0.50 (SEM)	3.9
Knobloch et al.[11]	Blood	11	342 ± 3.40 (SD)	454 ± 10.9 (SD)	1.3
Clarke[12]	Plasma	3	107	86	0.8

*The gradient is the ratio of cord-to-maternal serum riboflavin concentration.

Table II. Riboflavin concentration and protein binding in maternal and cord plasma

Patient	*Maternal (nmol/L)*	*Cord (nmol/L)*	*Gradient (C/M)**	*Binding (C/M)†*
1‡	103	189	1.8	0.76
2‡	104	153	1.5	0.82
3‡	204	260	1.3	—
4‡	138	154	1.1	0.93
5‡	128	139	1.1	0.96
6‡	89	185	2.1	1.0
7‡	106	189	1.8	0.96
8‡	92	94	1.0	1.1
9‡	114	171	1.5	1.1
10‡	95	137	1.4	0.95
11‡	73	81	1.1	—
12‡	114	234	2.1	—
13‡	107	246	2.3	—
14‡	63	121	1.9	—
15‡	100	106	1.0	1.3
16	154	139	0.90	—
17	188	161	0.86	0.99
18	102	135	1.3	0.96
19	111	135	1.2	1.0
20	76	140	1.8	—
Mean ± SD	113 ± 35	158 ± 47	1.45 ± 0.44	0.99 ± 0.13

The cord serum concentration is higher than the maternal concentration, with a p value of 0.001. Paired maternal and cord sera concentrations show, with few exceptions, a consistent difference that is not evident in protein binding.

*The gradient is the ratio of cord-to-maternal serum riboflavin concentration.

†The relative binding of riboflavin to cord and maternal serum was measured by equilibrium dialysis.

‡Vitamin supplement (3 mg/day riboflavin).

Previous studies of maternal and cord blood concentrations have yielded widely disparate results probably because of differences in analytic technique (Table I).

In the present study the riboflavin concentration was measured in maternal and cord sera at the time of delivery by an analytic technique based on more recently developed methods. High-performance liquid chromatography provides a specificity that was not previously possible, and analytic losses are corrected for by isotope dilution.

Material and methods

Riboflavin assay. The assays are performed in dim light because of the sensitivity of the riboflavin compounds. A blood sample is collected into a dry tube and permitted to clot on ice. It is centrifuged at 3000 rpm for 10 minutes and the serum is collected. The serum is frozen at −70° C until analyzed. Previous studies in our laboratory demonstrated that riboflavin, as identified by high-performance liquid chromatography, remains stable under these conditions for at least 7 months.

To the thawed sample add 0.1 ml ^{14}C-riboflavin (approximately 2500 cpm) to 0.5 ml serum. Radioactivity is measured in triplicate in 50 μl serum. Add 0.6 ml cold trichloroacetic acid, 20%, to the thawed sample and let stand at 0° C for 10 minutes. Centrifuge at 3000 rpm for 10 minutes, collect the supernatant, and neutralize with 0.1 ml 2.4 mol/L K_2HPO_4 buffer, pH 7.0. Inject 0.5 ml supernatant onto high-performance liquid chromatography and fractionate as described below. The eluted fractions are scanned in the fluorometer. The radioactivity (in counts per minute per milliliter) and fluorescence are measured in the riboflavin peak.

Recovery of radioactivity added to high-performance liquid chromatography was consistently >90%. Recovery of radioactivity through the entire analytic procedure varied from 85% to 90%.

Calculations

$$\frac{\text{Eluate (fluorescence)}}{\text{Riboflavin standard (fluorescence)}} \times \text{riboflavin standard (nmol/L)} = \text{eluate concentration (A)} \quad (1)$$

Correction for analytic losses

$$\frac{\text{Serum (cpm/ml)}}{\text{Eluate (cpm/ml)}} \times A = \text{riboflavin concentration (serum + } ^{14}\text{C-riboflavin) (B)} \quad (2)$$

Correction for added ^{14}C-riboflavin

$$B - \frac{\text{serum (cpm/ml)}}{^{14}\text{C-riboflavin (cpm/ml)}} = \text{Riboflavin concentration (nmol/L)} \quad (3)$$

The high-performance liquid chromatographic system has been described previously.[1] In this system, flavin adenine dinucleotide, flavin mononucleotide, and riboflavin elute at approximately 8.3, 9.3, and 10.3 minutes, respectively. Fluorescence is measured on an Aminco-Bowman spectrofluorometer (American Instrument Co., Silver Spring, Maryland) with excitation at 463 nm and emission at 520 nm. Radioactivity is assayed in a Packard Tricarb scintillation counter (Packard Instrument Co., Inc., Downers Grove, Illinois).

Material. Riboflavin, flavin mononucleotide, and flavin adenine dinucleotide were purchased from Sigma Chemical Co. (St. Louis, Mo.). The standard solutions are 26 nmol/L, 19.8 nmol/L, and 3.7 μmol/L, respectively. They are kept shielded from light with aluminum foil at 4° C and are stable for at least 24 hours. ^{14}C-2-D-riboflavin (53 mCi/mmol) was purchased from Amersham (Arlington Heights, Illinois). The purity of the compounds was checked by paper chromatography[1] and high-performance liquid chromatography.

Conversion to lumiflavin. Confirmation of the identity of the riboflavin peak obtained from serum was sought by exposing it to photolysis and comparing its effect to that of similarly exposed authentic riboflavin. The serum and authentic riboflavin were subjected to high-performance liquid chromatography as described above. The riboflavin peak was identified by elution time and fluorescence. This fraction was lyophilized and redissolved in 0.5 ml water and approximately 1000 cpm ^{14}C-riboflavin was added. The specific activity was measured (in counts per minute per milliliter per fluorescence unit). The pH was raised to 8 to 8.5 with 4N KOH. The serum sample and the riboflavin standard were transferred to glass tubes and simultaneously exposed to a 150 W white light at 18 inches for 2 hours. The tubes were covered to prevent evaporation. The pH was returned to neutral and 0.5 ml of each sample was rechromatographed on high-performance liquid chromatography, and the specific activity of the flavin derivative (elution time, 12.6 minutes) was measured.

Protein binding. Comparative binding of riboflavin by maternal and cord sera was measured by equilibrium dialysis. Paired samples of maternal and cord serum, 0.5 ml of each, were placed in individual dialysis bags and dialyzed together in a beaker against 10 ml Earle's buffered salt solution containing approximately 1000 cpm/ml ^{14}C-riboflavin. The beakers were shaken gently for 5 hours at 37° C in the dark. The concentration of radioactivity in the bags and the medium was determined at the end of incubation and percent binding was calculated.

$$\text{Binding (\%)} = \frac{\text{cpm/ml inside bag} - \text{cpm/ml outside}}{\text{cpm/ml inside}} \times 100$$

Subjects. Laboratory personnel served as control subjects for the determination of blood concentrations. Blood samples were drawn after a 2- to 3-hour fast. Only one subject (J. M. L.) took vitamin supplements.

Twenty mothers with uncomplicated pregnancies were selected for study. Mothers are routinely prescribed a multiple vitamin preparation (Stuartnatal, Stuart Pharmaceuticals, Wilmington, Delaware) at the first prenatal visit that contains a daily amount of 3 mg riboflavin. Five mothers were noncompliant and one patient (Table II, No. 15) discontinued supplementation 1 month before delivery. The remaining patients took vitamin supplements until within 24 hours of delivery. Blood samples were collected at delivery. Cord samples were drawn from double-clamped cord sections. The blood was placed on ice and permitted to clot for 15 minutes. The serum was separated by centrifugation at 3000 rpm for 10 minutes and frozen at −70° C until analysis.

Results

The mean serum concentration of plasma riboflavin in 12 apparently healthy subjects on a freely selected diet was 116 ± 46 nmol/L (SD). Notable was the broad range (48 to 222 nmol/L) without evident symptoms.

Five assays were performed on one serum sample (J. D.) over a period of several weeks. The average SD was 8%. Five additional samples were assayed more than once. In four samples the SD was 10% or less and in the fifth it was 17%.

To further confirm the specificity of the assay, the riboflavin peak obtained by high-performance liquid chromatography of the serum sample was derivatized by photolysis, and its major fluorescent product, lumiflavin, was measured as described in the Methods section. Six sera were studied. Fifty percent plus or minus seven percent of the riboflavin peak was recov-

ered as lumiflavin after repeat high-performance liquid chromatography. In five sera the difference in lumiflavin specific activity between authentic riboflavin and serum riboflavin was 8.6% ± 1.3% and in the sixth it was 20%. By this stringent test, >90% of the material in serum assayed as riboflavin was confirmed as riboflavin.

The plasma concentrations in the mothers at delivery were similar to those in the nonpregnant control population (113 ± 35 nmol/L [SD] versus 116 ± 46 nmol/L), with no significant statistical difference between the two groups. There was no detectable effect of vitamin supplementation in the test series. The plasma concentration in the mothers who had taken supplements was 109 ± 32.4 nmol/L versus 130 ± 51.4 nmol/L in the mothers who had not taken supplements ($p = 0.87$).

The cord plasma concentration was higher than the maternal concentration (158 ± 47 nmol/L [SD] versus 113 ± 35 nmol/L), with a p value <0.001 as determined by the double-tailed Student t test. Examination of paired samples revealed a consistent gradient toward the fetus (18 of 20 samples). The mean gradient was 1.45 ± 0.44. Comparison of maternal vein and umbilical vein levels between vitamin-supplemented and nonsupplemented groups showed insignificant differences in mean level and in the ratio of cord to maternal serum concentrations. The gradient was persistent and not significantly different for the supplemented versus unsupplemented groups. The ratio of the cord to the maternal serum concentration for the supplemented group was 1.54 ± 0.43, and for the unsupplemented group it was 1.23 ± 0.39.

Protein binding of riboflavin by maternal and cord plasma varied independently of the riboflavin concentration. The mean binding by 13 maternal plasmas was 60.8 ± 0.6 whereas that of the cord plasma was 59.0 ± 17. Inspection of the individual paired sera results confirmed the absence of differential binding by maternal and cord sera (Table II).

Comment

Riboflavin, or vitamin B_2, is a water-soluble vitamin. It is an essential cofactor in cellular oxidation. It is present in the body as riboflavin and its related coenzymes flavin adenine dinucleotide and flavin mononucleotide. Riboflavin is necessary for fetal growth and development. In experimental animals, riboflavin deficiency is associated with congenital malformations.[4] Smithells et al.[5] administered a multivitamin preparation, including riboflavin, to pregnant women in a controlled study and found a significantly reduced incidence of neural tube defects in the offspring.

Studies conducted in this laboratory by an in vitro perfusion technique demonstrated that riboflavin is actively transferred across human placenta, establishing a higher concentration in the fetal circulation than in the maternal circulation.[1] The driving force is a very rapid uptake of riboflavin from the maternal circulation.[2]

Several factors in addition to placental performance affect the in vivo concentrations of riboflavin. Prominent among these are the rates of metabolism and excretion of riboflavin by mother and fetus and binding to plasma protein. Therefore, one can not assume from the in vitro studies where these factors were not operative that a similar gradient would be found in vivo.

Four reports were found in the literature in which maternal and cord blood concentrations were measured. For each investigation a different analytic technique was used, and the results varied over a wide range (Table I). In three reports the cord concentration was higher than the maternal concentration, and in the fourth the reverse was found. It appeared timely to apply some of the newer analytic techniques to this question. High-performance liquid chromatography was used to isolate riboflavin from other circulating fluorescent compounds. Isotope dilution was used to correct for analytic losses.

A control study was done on a series of 12 adults. The serum riboflavin concentration was 116 ± 46 nmol/L (SD). The range was broad (48 to 143 nmol/L). The concentration was higher than that reported by two previous investigators using high-performance liquid chromatography. Ohkawa et al.[6] studied six subjects and reported a serum riboflavin concentration of 33 ± 2.2 nmol/L (SEM). Lambert et al.[7] reported a mean concentration of 25 nmol/L.

The following procedure was undertaken to confirm the specificity of our analytic technique. The riboflavin peak was collected after high-performance liquid chromatography in the standard assay technique, alkalinized, and exposed to light. On rechromatography, the riboflavin peak had disappeared and a new fluorescent peak, presumably lumiflavin, appeared, with an elution time of 12.6 minutes. By comparing the behavior of the serum "riboflavin" to authentic riboflavin, as described in the Methods section, it was concluded that >90% of the serum riboflavin had been identified correctly.

The analytic technique was used to measure the maternal and cord serum concentrations in a series of 20 pregnancies (Table II). The riboflavin concentration in the cord was 158 ± 47 nmol/L (SD) as compared with 113 ± 35 nmol/L in maternal serum, a statistically significant difference ($p = 0.001$). The concentration gradient was confirmed by examining paired maternal and cord sera, the mean gradient being 1.45 ± 0.44. Long-term vitamin supplementation did not affect serum riboflavin levels and did not contribute to the maternal-fetal gradient established by dietary intake alone in this population.

The perfusion studies had demonstrated that human placenta is capable of establishing a gradient of this magnitude. However, it was possible that differential binding of riboflavin to maternal and cord serum protein contributed to this effect. Specific binding of riboflavin to serum proteins has been reported.[3, 8] However, direct measurement of riboflavin binding by maternal and cord sera does not support the hypothesis. In maternal sera, 60.8% ± 16% (SD) of riboflavin was bound compared with 59.0% ± 17% in cord sera. Examination of the results from paired maternal and cord sera was confirmatory. The cord-to-maternal ratio of riboflavin binding was 0.99 ± 0.13 (Table II). It is clear that protein binding does not account for the transplacental gradient.

The mean fetal:maternal concentration ratio in serum in this series (1.45) closely approximates that observed in vitro in perfusion studies,[2] suggesting that similar mechanisms are operative. Synthesis of the in vitro and in vivo observations suggests the following description of the placental transport of riboflavin. Riboflavin is actively extracted from the maternal plasma, concentrated within the placenta, and transferred to the fetal circulation. The rate of transfer to the fetus exceeds the transfer rate in the reverse direction by a margin that satisfies fetal requirements and establishes a gradient.

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The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor

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The presence or absence of fetal breathing movements may be helpful in differentiating between true and false preterm labor. We attempted to demonstrate the clinical utility of this simple ultrasonic observation in predicting short-term delivery outcome during suspected preterm labor. A total of 50 pregnancies between 26 and 34 weeks' gestation with presumed preterm labor were observed in a prospective manner. During 20 minutes of observation with real-time ultrasound at the time of admission, fetal breathing movements were observed in 33 patients and considered absent in the remaining 17 patients. In those pregnancies with absent fetal breathing movements, true labor with subsequent delivery occurred in 16 patients. Of the 33 pregnancies with fetal breathing movements present, 29 continued for >48 hours. It appears that the absence of fetal breathing movement is a reliable indicator of imminent preterm delivery, irrespective of fetal membrane status ($p < 0.0001$). The observed mean sensitivity and specificity of this phenomenon in predicting short-term delivery outcome are 96.6% ± 3.3% (mean ± SD) and 80.0% ± 8.9%, respectively. A multivariant statistical model based on the frequency of contractions, white blood cell counts, initial cervical examination results, and premature rupture of membranes could successfully predict delivery outcome in 40% to 75% of cases. The addition of fetal breathing movement analysis to the model allowed for the correct prediction of outcome in 90% of the cases. (Am J Obstet Gynecol 1987;157:753-7.)

Key words: Fetal breathing movements, preterm labor, fetal membrane status

Fetal chest wall movements have been the subject of extensive experimentation and contemplation during the past decade since first described in fetal lambs by Dawes et al. in 1972.[1] Initially, Boddy and Dawes[2] reported that fetal breathing movements were present during parturition and that fetal hypoxia was associated with suppression of breathing, whereas intrauterine asphyxia induced gasping movements in the fetus. More recently, multiple investigators have documented that the cessation of fetal breathing movements is a normal physiologic event during term labor.[3-6] This phenomenon may be mediated by a direct effect of rising fetal prostaglandin levels on central fetal respiratory centers.[7-9]

Because an accurate prospective diagnosis of preterm labor can be difficult, any technique that will alert the clinician to true labor would be helpful. Recently in England, Castle and Turnbull[10] have demonstrated that fetal apnea in gestations less than 34 weeks' duration may be helpful in differentiating between true and false preterm labor, and subsequently predicting delivery outcomes. Although some investigators have been unsuccessful in attempts to replicate this observation,[11-13] more recent observations in term and preterm gestations by Boylan et al.[6] have supported this contention. Our prospective clinical study attempts to corroborate these previous findings and demonstrate the clinical utility of this simple ultrasonic observation in predicting preterm delivery outcomes at a university-based perinatal center.

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Material and methods

This prospective study involved 50 women with pregnancies between 26 and 34 weeks' gestation admitted to the University of Michigan labor and delivery unit with a presumptive diagnosis of preterm labor. All study participants had demonstrable uterine contractions on external tocographic monitors and/or demonstrable cervical changes before ultrasonic evaluation. Real-time ultrasound examinations by one of the authors (R. E. B.) and other senior obstetric residents were performed at the time of admission, and the presence or absence of fetal breathing movements was recorded for each patient. Fetal breathing movements were considered to be present if characteristic seesaw movements of the fetal chest or abdomen were sustained for at least 20 seconds. Fetal breathing movements were considered to be absent if no such fetal activity was noted during the 20-minute observation period. Most of these examinations were performed with a General Electric model RT-3000 real-time ultrasound unit.

The observer recorded the following data at the time of admission: presence or absence of fetal breathing

Table I. Association of fetal breathing with delivery outcome (n = 50)

	Delivery interval >48 hr	*Delivery interval <48 hr*
Fetal breathing movements		
Present	29	4
Absent	1	16
Sensitivity (mean ± SD) (%)		96.7 ± 3.3
Specificity (mean ± SD) (%)		80.0 ± 8.9
Positive predictive accuracy (mean ± SD) (%)		87.9 ± 5.7
Negative predictive accuracy (mean ± SD) (%)		94.1 ± 5.7

Fischer exact test = $p < 0.0001$.

Table II. Association of fetal breathing with delivery outcome (intact fetal membranes only; n = 40)

	Delivery interval >48 hr	*Delivery interval <48 hr*
Fetal breathing movements		
Present	26	4
Absent	1	9
Sensitivity (mean ± SD) (%)		96.3 ± 3.6
Specificity (mean ± SD) (%)		69.2 ± 11.8
Positive predictive accuracy (mean ± SD) (%)		86.7 ± 7.6
Negative predictive accuracy (mean ± SD) (%)		90.0 ± 7.6

Fischer exact test = $p < 0.0001$.

movements, biparietal diameter, femur length, presentation, glucose infusion, initial cervical examination results, duration of scan, frequency and strength of contractions, quality of examination, and presence of premature rupture of the membranes. A total of 10 patients had premature rupture of the membranes at the time of admission. The patient's subsequent clinical management was left to the discretion of the involved obstetric staff. Decisions to institute tocolysis, perform amniocentesis, administer antibiotics, or perform a cesarean delivery for progressive preterm labor and other obstetric indications were made on an individual basis.

The interval to delivery was recorded during a chart review. Data obtained from chart review included method of delivery, birth weight, Apgar scores, antibiotic use, tocolysis with ritodrine and/or magnesium sulfate, estimated fetal weight, smoking history, drug history, cervical cultures, white blood cell count, fetal heart rate, glucocorticoids, maternal age, parity, amniocentesis results, and any subsequent clinical diagnosis of chorioamnionitis or abruptio placentae.

Several statistical tests were used in the evaluation of these data. The two-way Fischer exact test was used to evaluate the hypothesis that the presence or absence of fetal breathing movements can predict short-term delivery outcome. The sensitivity and specificity of this diagnostic test were calculated in the standard manner. The χ^2 statistic and two-sample t tests were used to analyze the influence of various clinical parameters on the predictive value of fetal breathing movements. A model system to predict delivery outcome with and without the use of fetal breathing movement data was developed through the use of multivariant discriminate analysis.

Results

The relationship between the presence or absence of fetal breathing movements and imminent delivery in this study of presumed preterm labor is summarized in Table I. Fetal breathing movements were observed in 33 patients at the time of admission and considered absent in the remaining 17 patients. In those pregnancies without fetal breathing movements, true labor with subsequent delivery occurred in 16 patients. Of the 33 pregnancies with fetal breathing movements present, 29 continued for >48 hours. The hypothesis that the presence or absence of fetal breathing movements could successfully predict preterm delivery outcome was supported in 45 of 50 patients ($p < 0.001$). In the absence of premature rupture of membranes (n = 40), this phenomenon was still successful in predicting delivery outcome in preterm labor ($p < 0.0001$).

The mean (±SD) observed sensitivity and specificity of this diagnostic test in the study are 96.7% ± 3.3% and 80.0% ± 8.9%, respectively (Table I). The positive predictive accuracy was 87.9% ± 5.7%, while the negative predictive accuracy was 94.1% ± 5.7%. In the absence of premature rupture of membranes (n = 40), the sensitivity and specificity were 96.3% ± 3.6% and 69.2% ± 11.8%, respectively (Table II).

Our data suggest that several factors may contribute to the ability to observe fetal breathing in this clinical setting (Table III). The duration of scan ($p < 0.02$), white blood cell count ($p < 0.04$), smoking ($p < 0.03$), evidence of premature rupture of membranes ($p < 0.007$), and initial cervical examination results ($p < 0.007$) differed at reasonable levels of statistical confidence between pregnancies with and without fetal breathing movements. There was no statistical difference between the observation of fetal breathing versus fetal apnea in regard to intravenous glucose, frequency of contractions, or use of glucocorticoids. The correlation of premature rupture of membranes and initial cervical dilatation with fetal apnea may represent a potential observer bias in this study.

The correlation of several clinical parameters with a delivery interval of <48 hours confirms what the astute clinician already knows about predicting outcome in preterm labor (Table IV). Frequency of contraction

Table III. Comparison of clinical parameters by fetal breathing movement detection

	Fetal breathing movements present (n = 33)	*Fetal breathing movements absent (n = 17)*	*Statistical significance*
Maternal age (yr)	23.7 ± 8	25.3 ± 8	NS
Parity (%)			
0	34	64	$p < 0.09$
1	36	30	
2	27	06	
3	03	00	
Gestational age (wk)	30.6 ± 3.4	31.5 ± 2.8	NS
Biparietal diameter (mm)	77.5 ± 16	77.6 ± 13	NS
Femur length (mm)	57.9 ± 15	58.8 ± 17	NS
Duration of scan (min)	18.7 ± 1.6	22.4 ± 2.6	$p < 0.02$
Frequency of contractions (%)			
Every 1-5 min	51	64	NS
Every 5-10 min	45	24	
Every 10-15 min	04	12	
Intensity of contractions (%)			
Mild	27	30	NS
Moderate	66	53	
Strong	07	17	
Cervix (cm)	2.2 ± 0.7	3.2 ± 0.6	$p < 0.007$
Estimated fetal weight (gm)	1400 ± 300	1600 ± 270	NS
Fetal heart rate (bpm)	140 ± 30	140 ± 30	NS
Body temperature (°F)	98.1 ± 0.5	98.3 ± 0.6	NS
Premature rupture of membranes (%)	8	48	$p < 0.007$
White blood cell count (thousand/mm^3)	10.7 ± 7.0	13.4 ± 10.0	$p < 0.04$
Intravenous glucose (%)	84	94	NS
Smokers (%)	60	30	$p < 0.03$

Data are mean ± SD.

Table IV. Comparison of clinical parameters by interval to delivery

	>48 hr (n = 30)	*<48 hr (n = 20)*	*Statistical significance*
Maternal age (yr)	25.6 ± 6.0	23.4 ± 7.2	NS
Parity (%)			
0	36	55	NS
1	36	30	
2	23	15	
3	05	00	
Gestational age (wk)	30.7 ± 3.4	31.3 ± 3.1	NS
Biparietal diameter (mm)	78.8 ± 14	76.8 ± 15	NS
Femur length (mm)	58.3 ± 13	58.0 ± 16	NS
Duration of examination (min)	19.0 ± 3.5	21.5 ± 4.2	NS
Frequency of contractions (%)			
Every 1-5 min	56	55	$p < 0.04$
Every 5-10 min	44	30	
Every 10-15 min	0	15	
Intensity of contractions (%)			
Mild	24	35	NS
Moderate	66	55	
Strong	10	10	
Cervix (cm)	2.0 ± 0.5	3.4 ± 0.6	$p < 0.0001$
Estimated fetal weight (gm)	1400 ± 380	1500 ± 290	NS
Fetal heart rate (bpm)	140 ± 30	140 ± 30	NS
Body temperature (°F)	98.1 ± 0.5	98.2 ± 0.6	NS
Premature rupture of membranes (%)	6	40	$p < 0.003$
White blood cell count (thousand/mm^3)	10.4 ± 7.2	13.3 ± 9.1	$p < 0.02$
Antibiotics (%)	23	15	NS
Tocolysis (%)	63	65	NS
Intravenous glucose (%)	86	80	NS
Smokers (%)	60	35	$p < 0.08$
Cesarean delivery (%)	20	13	NS

Data are mean ± SD.

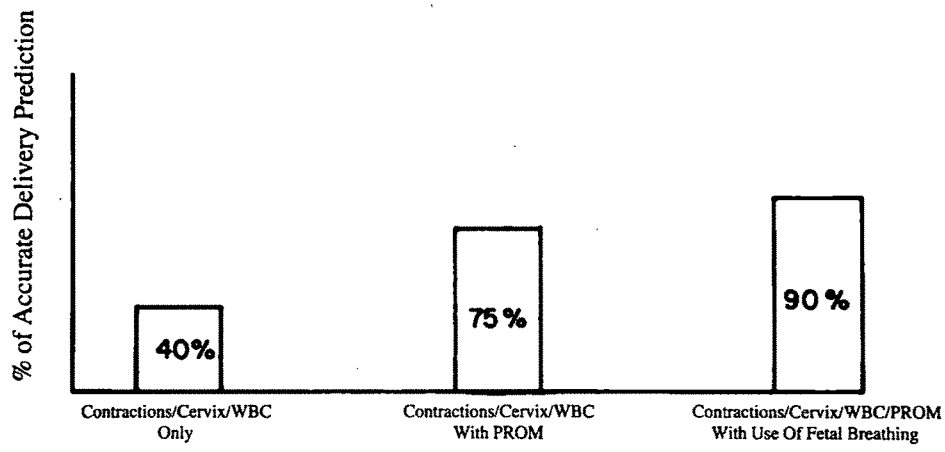


Fig. 1. Predictor of delivery outcome: A statistical model. *WBC* = White blood cell count; *PROM* = premature rupture of membranes.

($p < 0.04$), premature rupture of membranes ($p < 0.003$), initial cervical dilation ($p < 0.0001$), and white blood cell counts ($p < 0.02$) differed significantly at reasonable levels of statistical confidence between both outcome groups. Otherwise, maternal age, parity, gestational age, intravenous hydration, the use of antibiotics, and the method of delivery did not differ significantly in either outcome group. Interestingly, the use of pharmacologic tocolytic agents such as ritodrine and magnesium sulfate did not differ statistically between both outcome groups.

A statistical model was developed from a multivariant analysis of the most important discriminating variables in the prediction of delivery outcome in preterm labor (Fig. 1). Utilizing the clinical knowledge of the frequency of contractions, initial cervical dilatation, and white blood cell counts, the model predicted imminent delivery correctly in only 40% of the cases. Inclusion of premature rupture of membranes data increased diagnostic accuracy in our model to 75%. However, when fetal breathing movement analysis was used in addition to these data, the ability to predict imminent delivery increased to 90%. In the absence of premature rupture of membranes, the ability to predict imminent preterm delivery in this model appears to be dramatically improved with the use of fetal breathing movement analysis. When this model was applied to the diagnostic failures in this study, all five delivery intervals would have been predicted on the basis of simple clinical data without the use of fetal breathing movement analysis.

Comment

The prospective diagnosis of true preterm labor remains a major problem in obstetric practice. Difficulty in diagnosis inevitably results in some patients receiving treatment unnecessarily, with potential serious side effects. Likewise, other patients may not receive the full benefits of aggressive tocolysis unless properly identified as being at risk for preterm delivery. The practicing obstetrician could make use of any new diagnostic techniques that resolve this clinical dilemma.

The use of fetal breathing movements to differentiate prospectively between true and false preterm labor is a novel idea as first proposed by Castle and Turnbull.[10] Cessation of fetal breathing appears to be a normal physiologic event during labor at term[4-6] and during elective oxytocin induction of labor that results in delivery.[3] The notion that fetal apnea occurs during true preterm labor is less concrete. Several investigators have documented this phenomenon during preterm labor,[6, 14] while others have been unsuccessful in replicating this observation.[11-13]

The clinical utility of using the presence or absence of fetal breathing movements to predict imminent preterm delivery is clearly demonstrated in this study. Our data suggest that fetal apnea is a reliable prognostic indicator in preterm labor, with a negative predictive value of 94.1%. It would appear that fetal apnea during threatened preterm labor is a more reliable indicator of delivery outcome than documentation of fetal breathing. With a similar use of tocolytic agents in the two outcome groups, it appears that fetal apnea may still be predictive regardless of subsequent pharmacologic intervention. This indirectly suggests that fetal breathing movement analysis might be useful in identifying those pregnancies at risk to fail tocolytic therapy.

Central nervous system control of fetal breathing movements has been well documented in a variety of animal studies.[15] The phenomenon of fetal apnea during true labor has been attributed to a perceived direct effect of rising prostaglandin levels on central fetal respiratory centers.[15] When prostaglandin inhibitors are given to pregnant lambs, the frequency of fetal breathing movements was significantly increased[7]; similarly, the infusion of prostaglandin E_2 into pregnant lambs

results in the inhibition of fetal breathing.[8] The relationship between fetal breathing movements and prostaglandin levels in humans is poorly documented in humans. However, recent evidence that maternal serum prostaglandin metabolites are predictive of delivery outcome in preterm labor may provide indirect support of these contentions in humans.[16]

The major drawback to any clinical study involving short-term observation of fetal breathing movements is the inadequate sampling of a continuously occurring behavior.[17] Other potential errors include incorrect use of the gain setting, echo alterations by fetal and maternal movements, and poor choice of transducer placement.[17] The diagnostic accuracy of fetal breathing movement detection can be theoretically improved. Maternal inhalation of 3% carbon dioxide and subsequent maternal hypercapnia may increase the incidence of fetal breathing movements and improve our diagnostic accuracy in differentiating between true and false preterm labor.[18] Likewise, maternal glucose infusion may also increase the observed incidence of fetal breathing,[19,20] and thereby decrease the sampling error in the observation of this dynamic phenomenon. The fact that approximately 85% of our patients were receiving an intravenous glucose infusion during the ultrasonic examination may explain our observed accuracy of fetal breathing movement detection during the relatively short 20-minute observation period.

Our statistical model for predicting delivery outcome provides an interesting insight into the clinical dilemma of differentiating between true and false preterm labor. If the clinician uses only traditional data such as initial cervical examination results, white blood counts, frequency of contraction, and premature rupture of membranes to predict delivery outcome, a diagnostic accuracy only of 40% to 75% can be achieved. However, the addition of fetal breathing movement analysis to our model improves the diagnostic accuracy to 90%. Clearly, the additional use of fetal breathing movement analysis in the setting of threatened preterm labor, particularly in the absence of premature rupture of membranes, can be helpful to the clinician.

In conclusion, the use of fetal breathing movements to differentiate prospectively between true and false preterm labor is a novel idea with significant clinical utility for the practicing obstetrician. Although this phenomenon has been well documented in both term and preterm labor, the clinical importance of this noninvasive ultrasonic observation cannot be understated. The reliability and simplicity of fetal breathing movement analysis should allow for its widespread clinical use in the diagnosis and management of preterm labor.

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The significance of one abnormal glucose tolerance test value on adverse outcome in pregnancy

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A matched control study of 126 women equally divided into three groups (normal oral glucose tolerance test, one abnormal test value, and gestational diabetes mellitus) was undertaken to examine the relationships among oral glucose tolerance test results, glycemic control in pregnancy, and adverse perinatal outcome. Characterization of metabolic control for the one abnormal oral glucose tolerance test value and the gestational diabetes mellitus groups (before treatment) showed no significant difference. After the start of treatment, however, a significant ($p < 0.01$) difference between the groups in level of control was found. While no significant difference in the average birth weight between the three groups was discovered, the incidence of large infants (macrosomia and large for gestational age) was found to be significantly higher in the one abnormal oral glucose tolerance test group when compared with the normal (34% versus 9%; $p < 0.01$) and gestational diabetes mellitus group (34% versus 12%; $p < 0.01$). No significant difference for the incidence of an infant large for gestational age was found between the normal group and the patients with gestational diabetes mellitus after treatment. Neonatal metabolic disorders were found to be significantly higher for the one abnormal oral glucose tolerance test group (15%) when compared with the control and the gestational diabetes mellitus groups (3%). We conclude that, if left untreated, one abnormal value on an oral glucose tolerance test is strongly associated with adverse perinatal outcome. (Am J Obstet Gynecol 1987;157:758-63.)

Key words: Oral glucose tolerance test, gestational diabetes mellitus, metabolic control, perinatal outcome

It has become axiomatic that early diagnosis and treatment of gestational diabetes mellitus provide the best opportunity to prevent perinatal mortality and may provide an opportunity to reduce perinatal morbidity.[1-3] However, controversy has attended attempts to standardize the criteria for carbohydrate tolerance—the means by which gestational diabetes mellitus is diagnosed.[4-6] The best documented and most widely used diagnostic criteria for gestational diabetes mellitus are those of O'Sullivan and Mahan as modified for plasma values by the National Diabetes Data Group (1979) and later adopted by two international workshops,[7-10] which designated gestational diabetes mellitus as a unique entity defined as glucose intolerance with the first recognition or onset during pregnancy. Diagnosis of gestational diabetes mellitus requires at least two values to be abnormal on the oral glucose tolerance test. Evidence for the decision to base the diagnosis on two abnormal values remains unclear. Despite a large number of studies, questions continue to arise as to the efficacy of the current diagnostic criteria. Specifically, the significance of a single abnormal test value during an oral glucose tolerance test as a forecast for perinatal outcome is not defined. We therefore sought to investigate the hypothesis that women with one abnormal oral glucose tolerance test value, when not treated, are at a significantly higher risk for adverse outcome in pregnancy than are matched controls and women diagnosed and treated for gestational diabetes mellitus.

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Material and methods

A prospective, matched control study of three groups of women was designed. Group 1 consisted of patients with normal screening and oral glucose tolerance test values; group 2 contained patients with one abnormal test value; and group 3 consisted of patients with gestational diabetes mellitus with two abnormal test values.

Screening and diagnosis. Women were routinely screened between the twenty-fourth and twenty-eighth gestational weeks by use of a 50 gm oral glucose load with venous plasma samples taken at 1 hour. If the

Table I. Patient characteristics

	Group 1 (n = 42)	*Group 2 (n = 42)*	*Group 3 (n = 42)*
Age (yr)	27 ± 6	27 ± 6	28 ± 6
Parity (%)			
0	36	38	37
1-2	41	43	44
>3	23	19	19
Race (%)			
Black	38	37	37
Caucasian	62	63	63
Obesity/body mass index* (%)	19	19	20
Gestational age at testing (wk)	34 ± 4	34 ± 4	28 ± 2†

Data are mean ± SD. There were no significant differences between groups for all characteristics except age at testing.
*Body mass index = wt (kg)/ht $(m)^2$.
†Group 1 versus group 3, $p < 0.0001$; group 2 versus group 3, $p < 0.0001$.

results were ≥135 mg/dl, the patient was referred for a 3-hour oral glucose tolerance test. In preparation for the test, all patients were required to consume at least 150 gm carbohydrate for each of 3 days beforehand, with an overnight fast before the morning test. The oral glucose tolerance test consisted of ingesting, within 5 minutes, 100 gm oral glucose load; venous blood samples were drawn before the test and 1, 2, and 3 hours after ingestion. Plasma glucose was measured by Beckman analyzer, with abnormal values defined as follows: fasting, ≥105 mg/dl; 1 hour, ≥190 mg/dl; 2 hours, ≥165 mg/dl; and 3 hours, ≥145 mg/dl.[10]

Ambulatory glycemic control. To determine the ambulatory metabolic control of the subjects, a glucose profile was produced on the basis of self-monitored capillary blood glucose values. Subjects were instructed in the use of a memory-based reflectance meter. Instruction included skills evaluation for obtaining adequate capillary blood sample and testing. At each subsequent visit patients were reevaluated for self-monitoring skills. They were requested to test their blood glucose levels while fasting, before and after eating, and at bedtime. These values were offloaded onto a computer, providing accurate and reliable ambulatory glucose profiles. All group 2 (one abnormal oral glucose tolerance test value) and group 3 (gestational diabetes mellitus) subjects were asked to monitor capillary blood glucose for a 2-week baseline period after the test. For control subjects (group 1), every fourth patient was asked to monitor blood glucose during the baseline period. Patients with gestational diabetes mellitus continued to monitor their blood glucose throughout pregnancy.

Treatment of gestational diabetes mellitus. Diet treatment was calculated by consideration of body mass index before pregnancy. For obese subjects (index >27.3) 25 kcal/kg were prescribed; for normal subjects 30 kcal/kg were prescribed. Insulin treatment consisted of three injections per day divided into fasting (regular and intermediate), predinner (regular), and bedtime (intermediate) doses.

Table II. Oral glucose tolerance test results (mg/dl)

	Group 1	*Group 2*	*Group 3*
Fasting	84 ± 9	87 ± 12	93 ± 17*
1 hr	143 ± 27	183 ± 21†	190 ± 15
2 hr	122 ± 25	155 ± 24†	182 ± 22
3 hr	104 ± 22	105 ± 30	132 ± 34*

Data are mean ± SD.
*$p < 0.01$ versus groups 1 and 2.
†$p < 0.01$ versus group 1.

Neonatal outcomes. Physiologic measures during this investigation consisted of large for gestational age assessment, macrosomia, and metabolic abnormalities including hypoglycemia (blood glucose <40 mg/dl at birth reconfirmed by venous sample), hyperbilirubinemia (blood level >12 mg/dl), and polycythemia (central venous hematocrit >65%). Large infants were defined as birth weight either ≥4000 gm or ≥90% for a given gestational age.

Analysis. Univariate and bivariate statistical analyses were performed. Categorical data were evaluated with χ^2 analysis and Fisher's exact tests; continuous data were analyzed with Student's *t* test.

Results

Patient characteristics. One hundred twenty-six women were evenly divided between the three study groups. These subjects were drawn from among all women referred for an oral glucose tolerance test in 1985-1986. All groups were matched for race, maternal age, parity, and weight to remove any sampling biases. As shown in Table I, there was a significant difference in gestational week for time of the oral glucose tolerance test when group 1 (normal) and group 2 (one abnormal test value) were compared with group 3 (pa-

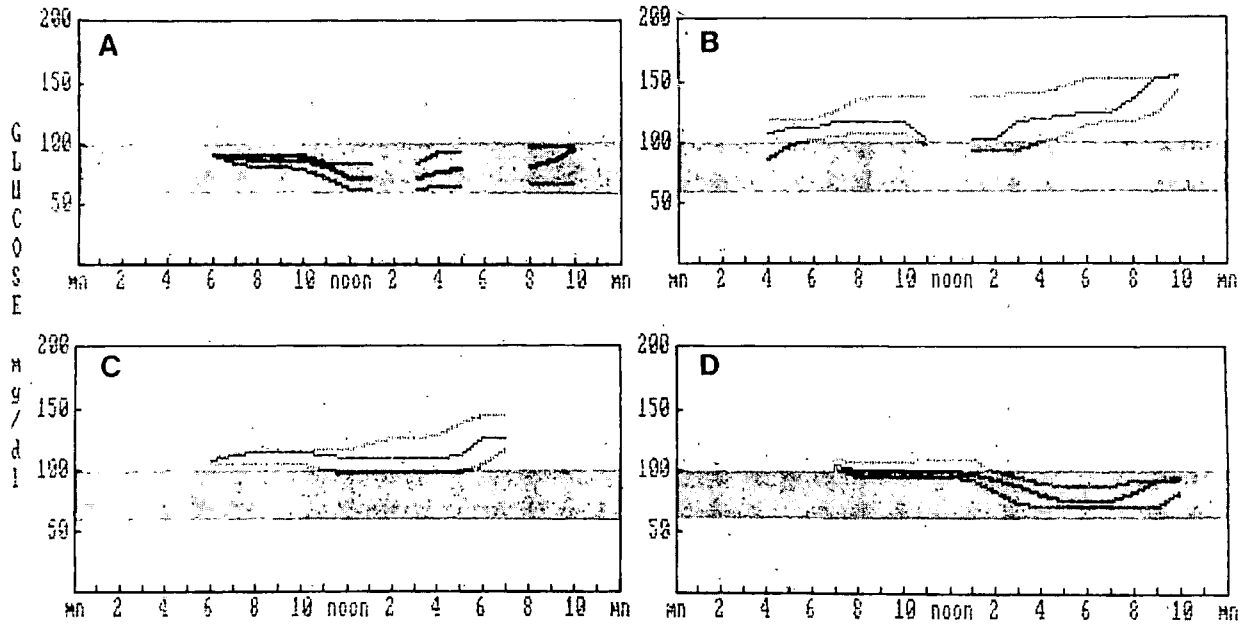


Fig. 1. Ambulatory glucose profiles of study patients. *A*, Two weeks of self-monitored glucose data collapsed into a single 24-hour typical day and shown as the twenty-fifth *(bottom)*, fiftieth *(middle)*, and seventy-fifth *(top)* percentile curves for a nondiabetic subject. Gaps in the curves show areas of insufficient self-testing. The *shaded area* identifies the target range for normal glycemia. *B*, The ambulatory glucose profile of a subject with gestational diabetes mellitus before treatment. *C*, The ambulatory glucose profile of a subject with one abnormal oral glucose tolerance test value. *D*, The ambulatory glucose profile of a treated subject with gestational diabetes mellitus.

Table III. Characterization of metabolic control (mg/dl) during 2-week periods (data from memory-base reflectance meters)

		Group 3		
	Group 2 (n = 42)	*Before treatment (n = 42)*	*In treatment (n = 42)*	*Significance**
Fasting	87 ± 12	95 ± 22	72 ± 22	$p < 0.002$
After breakfast	118 ± 33	121 ± 27	92 ± 26	$p < 0.0001$
Before lunch	110 ± 28	107 ± 35	85 ± 27	$p < 0.0001$
After lunch	128 ± 34	128 ± 31	94 ± 32	$p < 0.0001$
Before dinner	108 ± 31	110 ± 30	90 ± 33	$p < 0.01$
After dinner	121 ± 32	130 ± 29	98 ± 30	$p < 0.001$
Bedtime	126 ± 30	126 ± 36	100 ± 28	$p < 0.0001$

Data are mean ± SD.
*Group 2 versus group 3 in treatment.

tients diagnosed with gestational diabetes mellitus). Although no significant difference existed between groups 1 and 2, the latter were generally tested later. Within the 42 subjects in group 2, the earliest identification of one abnormal test value occurred for five patients during the twenty-ninth gestational week. Six additional patients were identified by 32 weeks' gestation, five more patients were discovered by 34 weeks' gestation, and the remainder were discovered between gestational weeks 35 and 39. Among these subjects, 67% were screened before the oral glucose tolerance test (the remainder were not screened, having been referred directly for the oral glucose tolerance test). Of those screened, 61% were found to have positive test results.

Oral glucose tolerance test profile of glucose metabolism. Characterizing groups 1 and 2 during the oral glucose tolerance test were significantly lower fasting plasma glucose levels than in the patients with gestational diabetes mellitus. Additionally, both 1- and 2-hour values were found to differ significantly between groups 1 and 2 (Table II). For group 2 the pro-

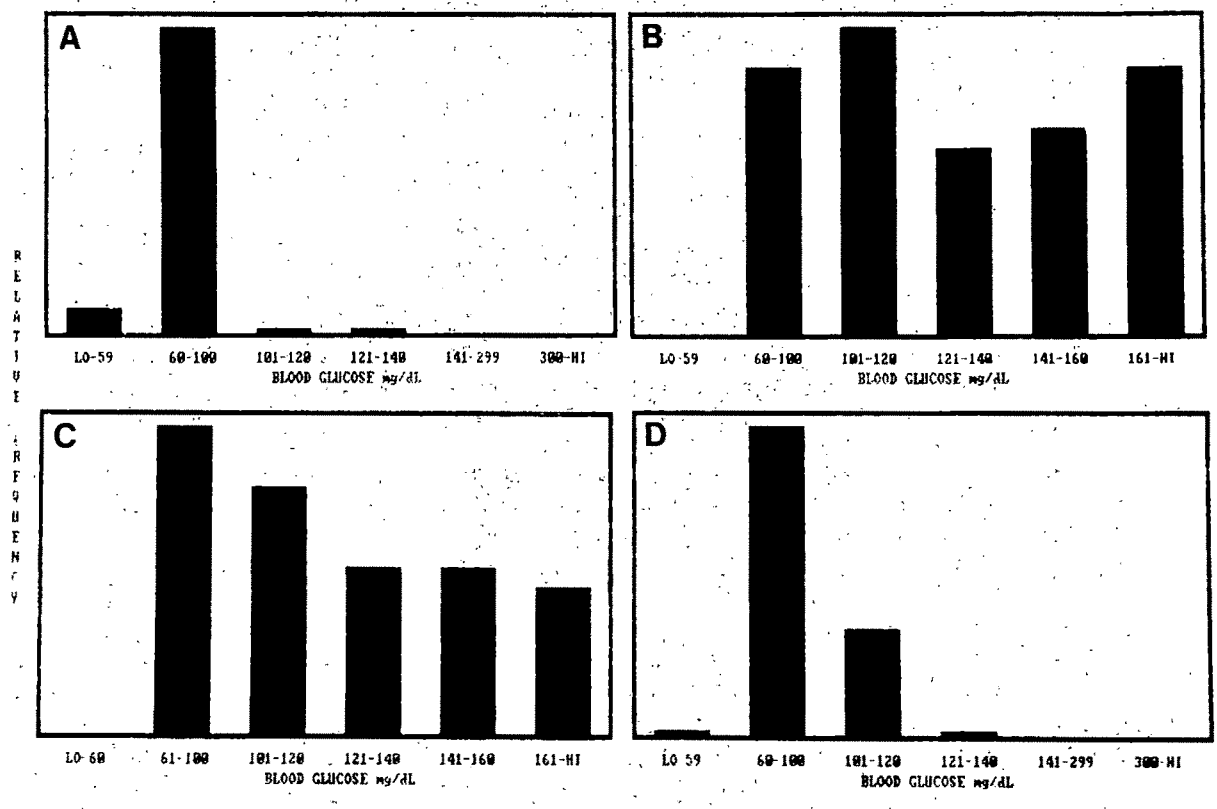


Fig. 2. Frequency distribution of blood glucose values from memory meters corresponding to the ambulatory glucose profiles. *A*, A normal patient. *B*, A patient with gestational diabetes mellitus before treatment. *C*, A patient with one abnormal oral glucose tolerance test value. *D*, A patient with gestational diabetes mellitus who is receiving treatment.

portion of patients with abnormal values favored the 1- and 2-hour periods (5% at fasting, 40% at 1 hour, 48% at 2 hours, 7% at 3 hours).

Ambulatory profile of glucose metabolism. Table III reflects the characterization of metabolic control for groups 2 and 3. Both groups tested their capillary blood glucose with the same frequency, 6.5 ± 1 test per day. A comparison by t test showed no significant difference between groups 2 and 3 before diet or insulin treatment. Within the gestational diabetes mellitus group, 26% were treated with insulin, while the remainder were placed on diet only regimens. No difference in overall metabolic control throughout pregnancy was found between the diet- and insulin-treated subjects. However, a significant difference was uncovered when group 2 patients (one abnormal value) and group 3 patients receiving treatment for gestational diabetes mellitus were compared.

Development of ambulatory glucose profiles. To represent the typical pattern of glycemia for the different groups, we produced ambulatory glucose profiles by use of the memory-based reflectance meter data. This profile represents 2 weeks of self-monitored blood glucose data collapsed into a single typical 24-hour period, represented as three smoothed curves (twenty-fifth, fiftieth, and seventy-fifth percentiles) of glucose values (Fig. 1).[11] There was a significant difference between group 1 *(A)* and both group 3 before treatment *(B)* and group 2 *(C)*. There was no significant difference in terms of average blood glucose and excursions between group 2 *(C)* and group 3 before the start of treatment *(B)*. However, a significant difference was found when the profiles for the group 3 patients treated for gestational diabetes mellitus *(D)* were compared with group 2 *(C)* and group 3 patients before treatment *(B)*. Finally, a comparison of profiles showed no significant difference between group 1 *(A)* and the group 3 patients treated for gestational diabetes mellitus *(D)*. Shown in Fig. 2 are the corresponding glucose values for each ambulatory glucose profile. The profile-derived glucose values for group 2 *(C)* were consistent with the ambulatory profile and reflected the significant difference in the oral glucose tolerance test 1- and 2-hour values when compared with group 1.

Neonatal outcome. No significant differences were found in the average birth weight between the three groups. However, the incidence of large infants (macrosomic and large for gestational age infants) was found

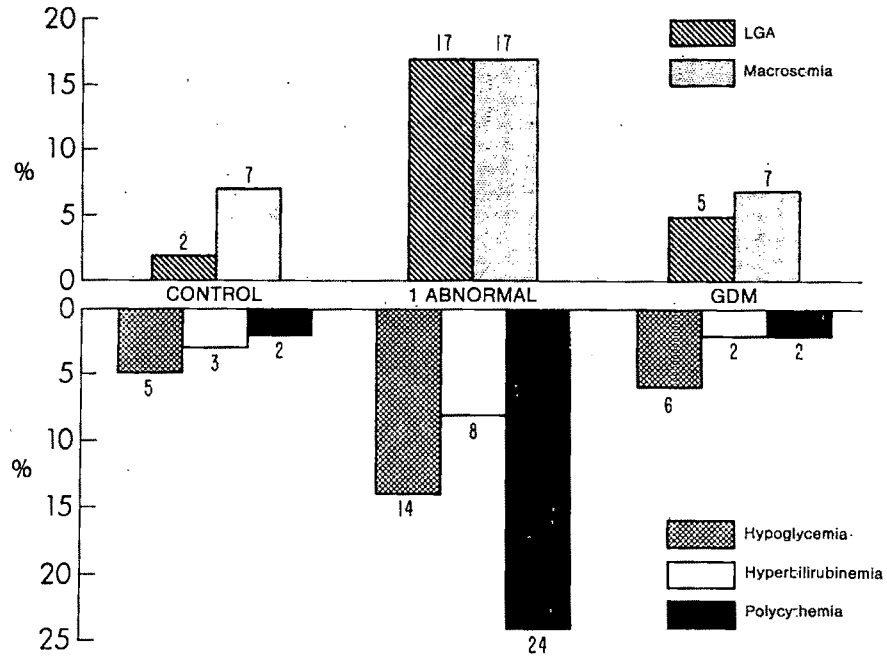


Fig. 3. Neonatal outcome, shown as the relative incidence of adverse neonatal outcomes for the three study groups. The numbers associated with each bar graph represent the actual percentages. *GDM* = Gestational diabetes mellitus.

to be significantly higher in group 2 when compared with groups 1 (34% versus 9%; $p < 0.01$.) and 3 (34% versus 12%; $p < 0.01$; Fig. 3). No significant difference for the incidence of large for gestational age infants was found between groups 1 and 3. Neonatal metabolic disorders were found to be significantly higher for group 2 (15%) when compared with groups 1 and 3 (3%; $p < 0.01$).

Comment

Our data suggest that one abnormal value on an oral glucose tolerance test should be regarded as a pathologic finding. We base this conclusion on three factors. First, we found a striking similarity between the subjects with gestational diabetes mellitus before treatment and those subjects with one abnormal oral glucose tolerance test value when we examined overall glycemic control (as represented by mean and SD). Second, when we used the ambulatory glucose profile as a method of analysis (to avoid the often misleading information from only the mean and standard deviation), we again found that the typical profile produced for gestational diabetes mellitus before treatment was indistinguishable from that of patients with one abnormal test value and significantly different from those for the treated patients with gestational diabetes mellitus. Using the four principal means of characterizing the ambulatory glucose profile (mean, average number of reversals in curve, average excursion after a reversal, and interquartile range), we were able to show that the patterns were both visually and statistically different from those of the treated patients with gestational diabetes mellitus. Third, the incidence of adverse neonatal outcome among the one abnormal test group was significantly higher than for the other groups (specifically, the incidence of large infants and metabolic abnormalities including polycythemia). We believe this high incidence of neonatal metabolic abnormalities was the result of poor glycemic control among the subjects with one abnormal test value. This is supported by previous studies of untreated patients with gestational diabetes mellitus that report an incidence of macrosomia between 20% and 30%,[12-14] a range consistent with the proportion of infants large for gestational age in the one abnormal test group. Therefore, we believe that this group should be considered as identical to group 3 patients who are not treated for gestational diabetes mellitus, with the same risk of adverse perinatal outcome.

Because the patients in the three groups were matched by age, weight, parity, and race, these findings have not been compromised by other factors that have been related to adverse fetal outcome. Furthermore, because our study included women whose one abnormal oral glucose tolerance test value was detected on the average at the thirty-fourth gestational week, these patients did not have undiscovered gestational diabetes mellitus as defined by existing diagnostic criteria.

Controversy still exists as to the criteria for diagnosis of gestational diabetes mellitus in terms of the actual glucose values. Recently, Tallarigo et al.[15] suggested that

even mild hyperglycemia may be associated with adverse outcome in pregnancy. Previously, Carpenter and Coustan[16] proposed stricter criteria for diagnosis of gestational diabetes mellitus. When we examined the 42 subjects in group 2 (one abnormal test value) and used the stricter criteria (95, 180, 155, and 140 mg/dl), 19 subjects would have been diagnosed as having gestational diabetes mellitus and 23 would have been considered normal. However, our analysis revealed that among these 23 "normal" subjects the incidence of adverse outcome was equivalent to that of the group who would have been diagnosed as having gestational diabetes mellitus according to the more strict criteria. Therefore our findings suggest that the criteria for diagnosis of gestational diabetes mellitus need to be modified. Furthermore, if treated, all patients with one abnormal oral glucose tolerance test value would have most probably experienced a significantly lower incidence of adverse perinatal outcome consistent with that found among individuals treated for gestational diabetes mellitus. We therefore conclude that these patients may have had gestational diabetes mellitus, and that the existing screening and diagnostic criteria need to be reexamined in light of these findings.

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Ultrasonic ratio of fetal thoracic to abdominal circumference: An association with fetal pulmonary hypoplasia

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Real time ultrasound was used in pregnant women who were considered to be at risk for development of pulmonary hypoplasia (n = 26). The population consisted of women who had either premature rupture of membranes (n = 16) or oligohydramnios (n = 10). The ratio calculated by comparing the fetal thoracic circumference to abdominal circumference was obtained in all cases within 10 days of delivery. This ratio correlated strongly with neonatal outcome. Multivariate regression models of neonatal outcome were developed to analyze the relationship of gestational age at which premature membrane rupture occurred, the duration of premature rupture, date of birth, and prenatal diagnosis. The addition of the thoracic circumference to the abdominal circumference ratio increased significantly the proportion of explained variability. Application of this ratio should be considered in evaluating fetal status in oligohydramnios and in the expectant management of premature rupture of membranes. (AM J OBSTET GYNECOL 1987;157:764-9.)

Key words: Pulmonary hypoplasia, premature rupture of membranes, oligohydramnios

The presence of pulmonary hypoplasia has been well documented in both renal and nonrenal origins of decreased amniotic fluid.[1,2] The antepartum diagnosis of developing pulmonary hypoplasia would be beneficial in the management of pregnancies with these complications. Callan et al.[3] have established a normal ultrasonogram for absolute fetal thoracic circumference and for the ratio of thoracic circumference to abdominal circumference. The ratio (thoracic circumference to abdominal circumference) has been reported to remain constant throughout pregnancy (0.94 ± 0.05). An earlier report[4] noted a serial decline in thoracic circumference to abdominal circumference, with the subsequent development of pulmonary hypoplasia.

Our study was done to test the significance of thoracic circumference to abdominal circumference, compared with other parameters in predicting fetal lung growth as manifested by neonatal respiratory function.

Material and methods

The population consisted of patients who experienced premature rupture of membranes at <28 weeks of gestation, duration ≥7 days (n = 16), or patients who experienced oligohydramnios with intact membranes, duration >7 days (n = 10).

Each patient had at least one real time ultrasound examination with a linear array probe (Sonoline SL, Siemens, Erlanen, West Germany; Toshiba SAL 20, Otawara, Japan; Acuson, Palo Alto, California) at 3.5 MHz within 10 days of delivery. The thoracic circumference was measured in the transverse plane of the fetus at the level of the four-chamber view of the heart (Fig. 1). The abdominal circumference was measured in the transverse plane of the fetus at the level of the stomach (Fig. 2). Neonatal assessment was blinded to the ratio of thoracic circumference to abdominal circumference.

Neonatal outcome was assigned to one of four groups on the basis of clinical profile, pulmonary manifestations, and the presence or absence of autopsy, as follows:

Group 1. Neonatal death associated with pulmonary hypoplasia. Neonatal death occurred within 2 days of birth. All these neonates were originally in group 2 until pulmonary hypoplasia was confirmed by autopsy.

Group 2. Clinical diagnosis of pulmonary hypoplasia. There was evidence of respiratory failure and abnormally high pulmonary morbidity in this group. A tentative diagnosis of pulmonary hypoplasia was made on the basis of the following: (a) clinical respiratory disease that did not conform to the classic model of hyaline membrane disease; (b) chest radiographic evidence of small lung volume, with the diaphragm elevated above the seventh rib, downward sloping ribs, and a bell-shaped thoracic cage; (c) ventilatory pressure on inflation of >25 cm water with an alveolar-arterial oxygen gradient of >600 mm Hg; (d)

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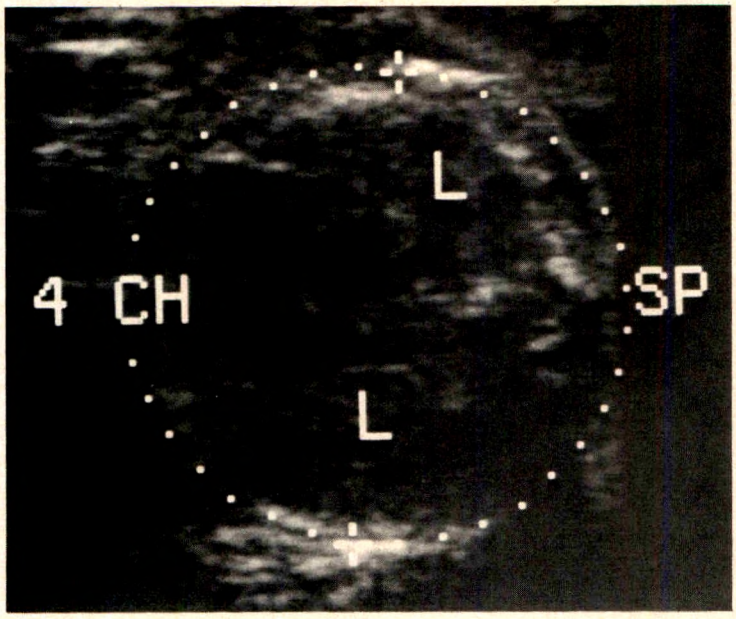


Fig. 1. Linear array ultrasound photograph showing a transverse section of the thoracic circumference with fetal spine (*SP*), four chamber heart (*4 CH*), and lung (*L*).

presence of interstitial pulmonary or pleural air leakage; and (e) any evidence of right-to-left intracardiac shunt.

Group 3. Benign outcome. This group consisted of neonates who experienced transient respiratory failure (<1 day) and whose clinical course was indistinguishable from that of hyaline membrane disease or pneumonia.

Group 4. Asymptomatic outcome.

The data initially were analyzed by the Student's *t* test. A multivariate statistical analysis of the relationship between thoracic circumference to abdominal circumference and neonatal outcome was implemented. For this analysis an ordinal variable was constructed to reflect neonatal outcome (1 = neonatal death with pulmonary hypoplasia; 2 = clinical diagnosis of pulmonary hypoplasia; 3 = benign neonate; 4 = asymptomatic neonate).

Pearson correlation coefficients were computed to determine the relationship of thoracic circumference to abdominal circumference to gestational age at rupture; duration of rupture; age at birth; absolute thoracic circumference; and absolute abdominal circumference. A multiple regression model was used to measure the partial correlation between thoracic circumference to abdominal circumference and neonatal outcome, controlling for duration, age at onset of premature rupture of membranes, and age at birth. The purpose of this analysis was to test the hypothesis that the ratio of thoracic circumference to abdominal circumference affords information about neonatal outcome over and above that afforded by duration of premature rupture of membranes, age at onset of premature rupture of membranes, and age at birth.

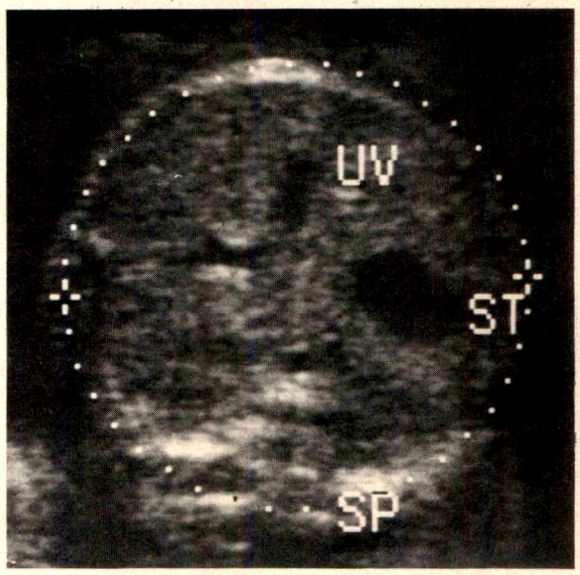


Fig. 2. Linear array ultrasound photograph showing a transverse section of the abdominal circumference with the fetal spine (*SP*), stomach (*ST*), and umbilical vein (*UV*).

Data of the premature rupture of membranes and oligohydramnios cases were then combined. A regression model was constructed for the ratio of thoracic circumference to abdominal circumference, age at birth, and indication (premature rupture of mem-

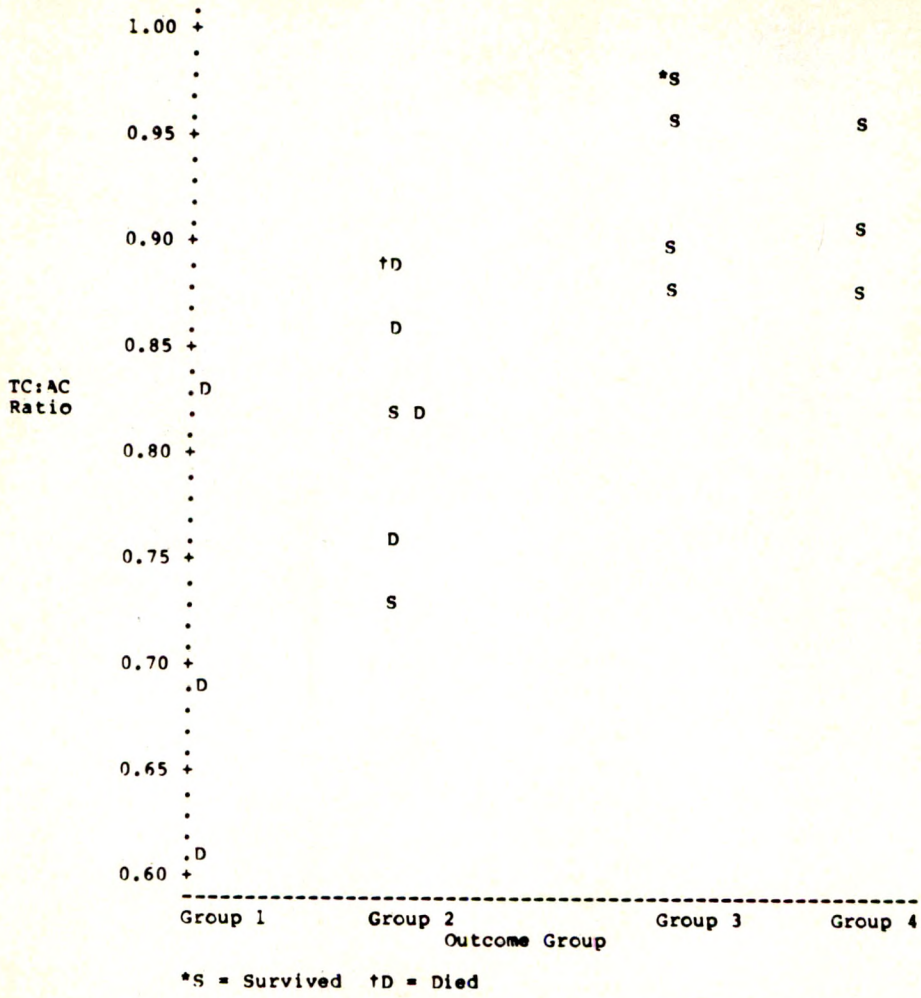


Fig. 3. The thoracic circumference to abdominal circumference ratio in fetuses seen for the indication of premature rupture of membranes as compared with neonatal outcome.

Table I. Neonatal autopsy findings with fetal thoracic circumference to abdominal circumference ratio and prenatal diagnosis

Case no.	*Prenatal diagnosis*	*TC to AC*	*Autopsy findings*
2	P	0.61	Pulmonary hypoplasia, intraventricular hemorrhage, renal vein thrombosis
5	P	0.83	Oligohydramnios tetrad (nonrenal), including pulmonary hypoplasia
6	P	0.69	Oligohydramnios tetrad (nonrenal), including pulmonary hypoplasia
10	O	0.81	Potter's syndrome, hydrocephalus
12	O	0.88	Multiple congenital anomalies, pulmonary hypoplasia
13	O	0.86	Hypoplastic bladder, pulmonary hypoplasia
14	P	0.76	Hyaline membrane disease, respiratory insufficiency, congestive heart failure
15	O	0.74	Charge syndrome, renal agenesis, pulmonary hypoplasia
16	O	0.72	Multiple congenital anomalies, cystic kidneys, pulmonary hypoplasia
18	P	0.89	Hyaline membrane disease, respiratory insufficiency, pneumonia
19	P	0.86	Hyaline membrane disease, bronchopulmonary dysplasia
20	O	0.94	Respiratory distress syndrome, severe intrauterine growth retardation, pulmonary hemorrhage
21	O	0.87	Polycystic kidney disease, pulmonary hypoplasia
22	P	0.82	Hyaline membrane disease, bronchopulmonary dysplasia, patent ductus arteriosus, intraventricular hemorrhage
24	O	0.75	Multiple congenital anomalies, pulmonary hypoplasia, isodicentric chromosome 13
27	O	0.73	Pulmonary hypoplasia, renal dysplasia

P = Premature rupture of membranes; O = oligohydramnios with intact membranes.

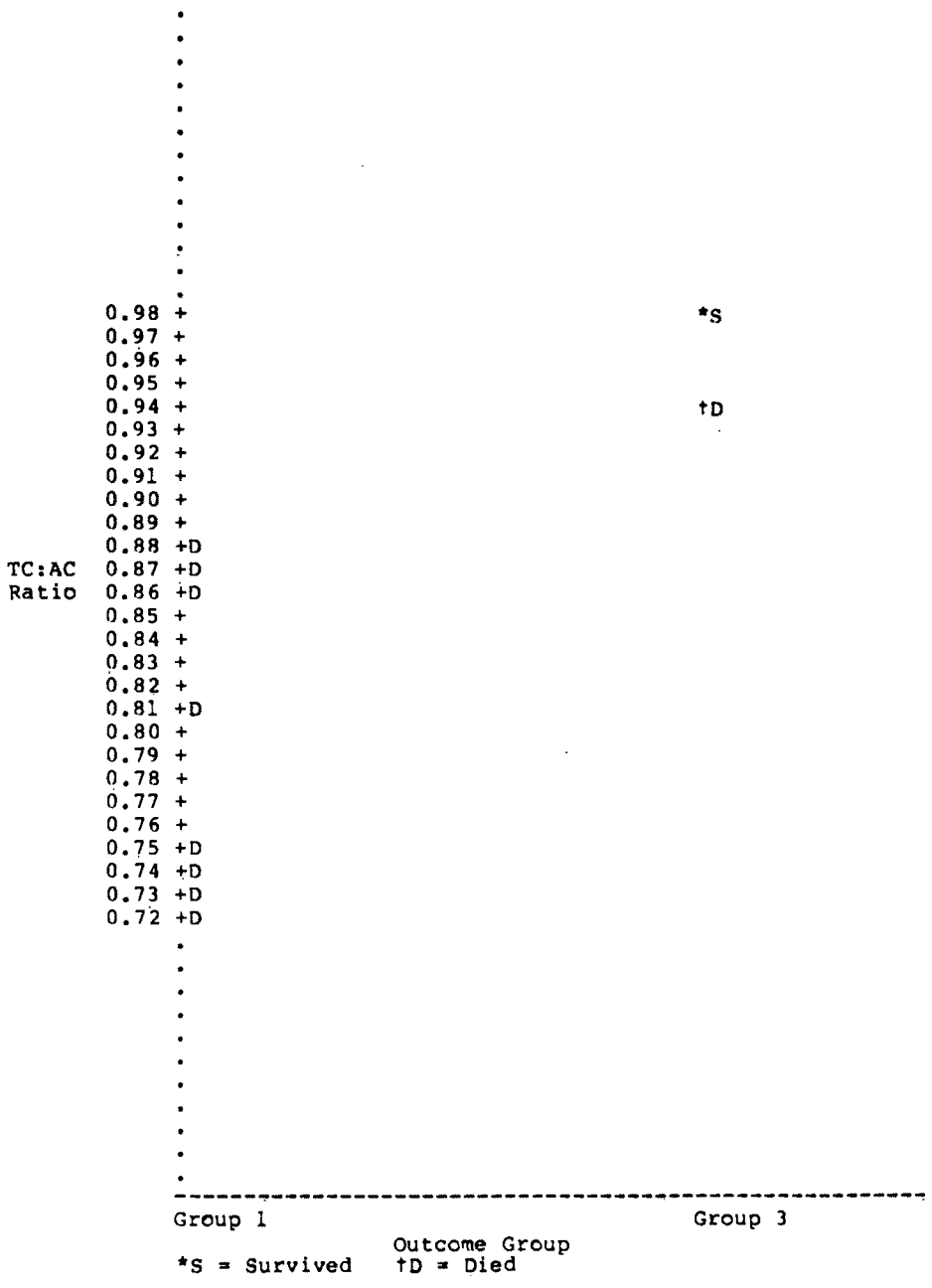


Fig. 4. The thoracic circumference to abdominal circumference ratio in fetuses seen for the indication of oligohydramnios as compared with neonatal outcome.

branes versus oligohydramnios). A partial correlation coefficient was then constructed for the combined indications.

Results

The ratio of thoracic circumference to abdominal circumference was associated with neonatal outcome. The lower thoracic circumference to abdominal circumference ratios were seen in the fetuses who later developed pulmonary hypoplasia (Figs. 3 and 4). All fetuses with thoracic circumference to abdominal circumference ratios >0.89 had either a benign or asymptomatic neonatal course. Of the 26 neonates alive at birth, 62% (n = 16) died. Table I lists the autopsy findings in each case. Group 1 accounted for 69% (n = 11), group 2 for 25% (n = 4), and group 3 for 6% (n = 1).

Table II compares the thoracic circumference to abdominal circumference ratio in premature rupture of membranes for the following parameters: death versus survival; group 1 versus groups 2, 3, and 4; and combined groups 1 and 2 versus groups 3 and 4. The difference in the thoracic circumference to abdominal circumference ratio was significant.

Table III compares the combined groups 1 and 2

Table II. Thoracic circumference to abdominal circumference ratio in premature rupture of membranes: Comparison of thoracic circumference to abdominal circumference ratio in 16 cases of premature rupture of membranes for death, autopsy-proved pulmonary hypoplasia, and clinical pulmonary hypoplasia

	N	*Mean*	*SD*	*Minimum*	*Maximum*	
Death	7	0.78	0.10	0.61	0.89	$p < 0.03$
Survived	9	0.89	0.08	0.73	0.98	
Group 1*	3	0.71	0.11	0.61	0.83	$p < 0.01$
Groups 2†, 3‡, 4§	13	0.87	0.08	0.73	0.98	
Groups 1, 2	9	0.78	0.09	0.61	0.89	$p < 0.01$
Groups 3, 4	7	0.92	0.04	0.88	0.98	

*Group 1 = autopsy-proved pulmonary hypoplasia.
†Group 2 = clinical diagnosis of pulmonary hypoplasia.
‡Group 3 = benign respiratory disease.
§Group 4 = asymptomatic.

Table III. Comparison of neonatal outcome in 16 cases of premature rupture for duration of ruptured membranes, gestational age at onset of rupture, and gestational age at birth

	n	*Mean*	*SD*	*Minimum*	*Maximum*	
Premature rupture of membranes duration (days)						
Groups 1,* 2†	9	45.9	28.9	12	112	$p < 0.05$
Groups 3,‡ 4§	7	20.7	13.9	7	42	
Gestational age at onset (wk)						
Groups 1, 2	9	22.4	4.4	12	27	$p < 0.02$
Groups 3, 4	7	26.6	1.3	25	28	
Date of birth						
Groups 1, 2	9	29.3	2.2	27	34	NS
Groups 3, 4	7	29.9	2.5	26	34	

*Group 1 = autopsy-proved pulmonary hypoplasia.
†Group 2 = clinical diagnosis of pulmonary hypoplasia.
‡Group 3 = benign respiratory disease.
§Group 4 = asymptomatic.

Table IV. Pearson correlation coefficients between neonatal outcome, thoracic circumference to abdominal circumference ratio, gestational age at onset of rupture, duration of rupture of membranes, absolute thoracic circumference, and absolute abdominal circumference in 16 cases of premature rupture of membranes

	*Outcome**	*TC to AC*
Thoracic circumference to abdominal circumference ratio	0.75 ($p < 0.001$)	1.0
Gestational age at premature rupture of membranes	0.71 ($p < 0.002$)	0.58 ($p < 0.02$)
Duration of premature rupture of membranes	−0.55 ($p < 0.03$)	−0.56 ($p < 0.02$)
Thoracic circumference (absolute)	0.69 ($p < 0.003$)	0.66 ($p < 0.003$)
Abdominal circumference (absolute)	0.18 (NS)	−0.06 (NS)

NS = Not significant.
*Values of outcome variables: 1 = neonatal death with pulmonary hypoplasia; 2 = clinical diagnosis of pulmonary hypoplasia; 3 = benign neonates; 4 = asymptomatic neonates.

versus groups 3 and 4 for the following parameters: duration of premature rupture of membranes, gestational age at premature rupture of membranes, and date of birth. The difference in duration and gestational age at onset of premature rupture of membranes was significant.

Table IV lists the correlation coefficients between the thoracic circumference to abdominal circumference ratio, outcome, gestational age at premature rupture of membranes, duration of premature rupture of membranes, absolute thoracic circumference, and absolute abdominal circumference. The thoracic circumference to abdominal circumference ratio correlated strongly with outcome ($r = 0.75$, $p < 0.001$).

The multivariate regression model of outcome in premature rupture of membranes, on the basis of age at onset, duration, and date of birth was significant ($R^2 = 51\%$, $p < 0.03$). The addition of the thoracic circumference to abdominal circumference ratio to the model increased the proportion of explained variability ($R^2 = 73\%$, $p < 0.01$). The partial correlation between the thoracic circumference to abdominal circumference ratio and outcome was 44%, indicating that 44% of variability in the regression model is accounted for by the inclusion of the thoracic circumference to abdominal circumference ratio.

The multivariate regression model of outcome, on the basis of age at birth and indication (premature rupture of membranes or oligohydramnios), was developed ($R^2 = 31\%$, $p < 0.02$). The addition of the thoracic circumference to abdominal circumference ratio to the model increased the proportion of explained variability ($R^2 = 68\%$, $p < 0.001$). The partial correlation between the thoracic circumference to abdominal circumference ratio and outcome was 54%, indicating that 54% of variability in the model is accounted for by the inclusion of the thoracic circumference to abdominal circumference ratio.

Correlation coefficients for oligohydramnios were not calculated. In these neonates, 89% were in group I ($n = 8$). The presence of congenital anomalies was noteworthy, including pulmonary hypoplasia, in all cases of oligohydraminos with a low thoracic circumference to abdominal circumference ratio.

Comment

Earlier reports have documented the significance of gestational age at onset of premature rupture of membranes and duration of premature rupture of membranes in the development of pulmonary hypoplasia.[5, 6] Similar findings were shown in this study. There was a correlation between neonatal outcome and duration of premature rupture of membranes ($r = 0.55$, $p < 0.03$). Also, the gestational age at onset of premature rupture of membranes correlated with neonatal outcome ($r = 0.58$, $p < 0.01$). Nimrod et al.[7] reported on the association of pulmonary hypoplasia and a low thoracic circumference. This study is in agreement with those findings by Nimrod et al. Thoracic circumference correlated strongly with outcome ($r = 0.69$, $p < 0.003$), but the thoracic circumference to abdominal circumference ratio correlated more strongly with outcome ($r = 0.75$, $p < 0.001$). No additional information was afforded by gestational age at birth.

The oligohydraminos group was not analyzed for significance of thoracic circumference to abdominal circumference because of the small sample size and the absence of information on onset and duration of oligohydramnios.

Decreased amniotic fluid is associated with pulmonary hypoplasia. Our study suggests that the thoracic circumference to abdominal circumference ratio affords valuable information in addition to duration of premature rupture of membranes, gestational age at onset of premature rupture of membranes, date of birth, absolute thoracic circumference, and absolute abdominal circumference.

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Effects of alterations of zinc-to-phosphorus ratios and meconium content on Group B *Streptococcus* growth in human amniotic fluid in vitro

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Several authors have described the bacterial growth-altering properties of amniotic fluid. We examined Group B *Streptococcus* growth in aseptically obtained amniotic fluid in vitro after altering its zinc, phosphorus, and meconium contents. Zinc and phosphorus levels were calculated in amniotic fluid and in meconium. Separate solutions of zinc and phosphorus were added to yield concentrations of 0.7, 7.0, 70, and 700 μmol. The solutions were incubated with Group B *Streptococcus* III 893 and *Escherichia coli* C5 strains, and 24-hour growth curves were plotted. Meconium, 0.5 mg/ml, was added to each amniotic fluid + zinc and amniotic fluid + phosphorus solution, and growth curves were plotted. The rate of proliferation of Group B *Streptococcus* varied directly with the zinc concentration (700 = 70 > 7 > 0.7 μmol) and inversely with the phosphorus content (700 < 70 < 7 = 0.7 μmol). Meconium enhanced the proliferative effect of zinc and hindered the inhibitory effect of phosphorus. Thus one possible mechanism whereby meconium enhances bacterial growth in amniotic fluid may be by alteration of zinc-to-phosphorus ratios. (AM J OBSTET GYNECOL 1987;157:770-3.)

Key words: Group B *Streptococcus* growth, amniotic fluid, zinc-to-phosphorus ratios, meconium content

The effect of amniotic fluid on bacterial growth in vitro has been studied extensively. However, the literature reports discrepant results regarding the effect of differing zinc and phosphate concentrations on the growth-inhibiting properties of the fluid. Schlievert et al.[1] described a low molecular weight peptide zinc complex, which acted as a bacterial growth inhibitor and was inactivated by phosphate. However, Blanco et al.[2] demonstrated the inhibition of Group B *Streptococcus* growth in amniotic fluid that contained increasing concentrations of phosphate. In a prior study,[3] we showed strain-specific differences in the in vitro growth patterns of Group B *Streptococcus* type III. A major purpose of the present study was to assess the effects of alterations in zinc-to-phosphorus ratios on strain-specific growth rates of Group B *Streptococcus* type III.

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Previous workers have also described the growth-supporting properties of amniotic fluid in the presence of meconium. Florman and Teubner[4] have demonstrated the enhancement of growth of *Escherichia coli, Listeria monocytogenes*, and *Staphylococcus aureus* after the addition of meconium to amniotic fluid. They showed a striking increase in the number of organisims per milliliter in these fluids. Meis et al.[5] reported an association between the early passage of heavy meconium and amnionitis. A second purpose of this work was to study the in vitro growth patterns of Group B *Streptococcus* type III in amniotic fluid that contains meconium.

Material and methods

Sterile amniotic fluid specimens were obtained by amniocenteses performed during the second or third trimesters for chromosome or lung maturity studies, respectively. All patients were free of infection and were not taking any medications. Bloody, meconium-stained, or infected fluid was excluded. Each specimen was filtered through a 0.45 μm filter to remove cellular debris and then cultured to ensure sterility. All second-trimester amniotic fluid specimens were grouped into a single pool as were all third-trimester specimens. Zinc levels were measured after wet digestion with nitric-sulfuric acid, followed by measurement by atomic absorption at 214 nm in an air acetylene flame with a

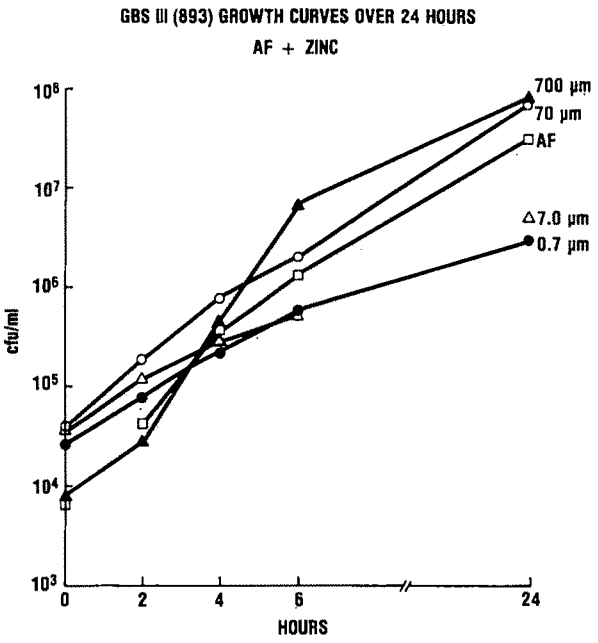


Fig. 1. The 24-hour growth curves of Group B *Streptococcus* III (893) in amniotic fluid and after the addition of zinc in varying concentrations.

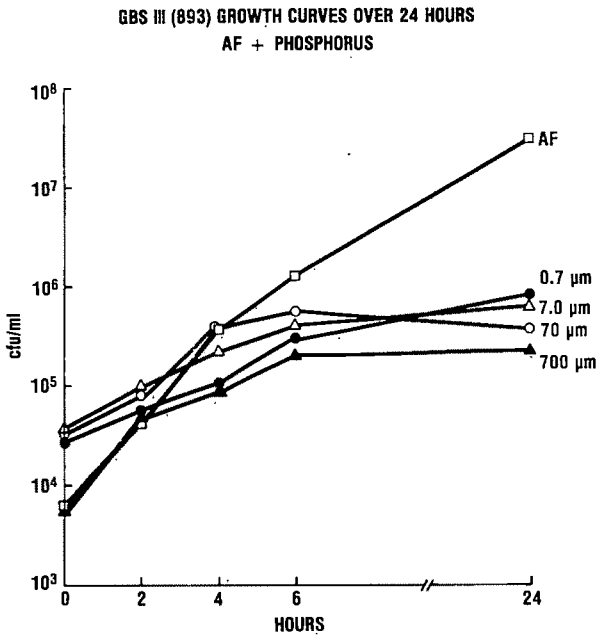


Fig. 2. Group B *Streptococcus* III (893) growth curves over 24 hours in amniotic fluid and after the addition of phosphorus in varying concentrations.

Perkin-Elmer atomic absorption instrument, model 603. Extensive precautions were required to avoid contamination of the samples by atmospheric dust, a common source of traces of zinc. All glassware was extensively washed with acid before use. Phosphorus was measured by the method of Lopez and Lowry and modified to measure phosphorus in the lowest ranges. Zinc-to-phosphorus ratios were calculated. Sterile solutions that contained zinc chloride were added to amniotic fluid aliquots in amounts that yielded the following concentrations of zinc: 0.7, 7.0, 70, and 700 μmol. These values were calculated to make molar solutions on the basis of the molecular weight of zinc. Sodium phosphate was added in amounts to yield concentrations of phosphorus 0.7, 7.0, 70, and 700 μmol. Aseptically obtained meconium was analyzed for its zinc and phosphorus contents, and a standard amount (0.5 mg/ml) was added to the amniotic fluid solutions that contained the above mentioned concentrations of zinc or phosphorus. Paired 2 ml aliquots of amniotic fluid were inoculated at zero time with approximately 1×10^4 colony-forming units per milliliter of a known virulent strain of Group B *Streptococcus* type III (893) or with *E. coli* (C5). Inocula were prepared by growing the bacteria to the log phase in Todd Hewitt broth, then adjusting the concentration with normal saline solution to approximately 1×10^3 colony-forming units per milliliter. Twenty microliters of the saline Group B *Streptococcus* or *E. coli* suspension were inoculated into the solutions previously prepared. The specimens were mixed well, aliquots removed for quantitative cultures, and incubation begun at 37° C. Two milliliter aliquots of Todd-Hewitt broth and of normal saline solution were used as positive and negative controls, respectively. Duplicate 10 μl aliquots were removed at 2, 4, 6, and 24 hours from each specimen. Quantitative cultures were performed by successive tenfold dilutions of each sample, followed by inoculation of a measured volume of each dilution onto Columbia sheep blood agar plates. After overnight incubation at 37° C, colony counts were performed.

Results

The zinc and phosphorus levels in the amniotic fluid were 192 and 0.90 μg/ml, respectively. The zinc-to-phosphorus ratios were >200, which is in the noninhibitory range, as described by Schlievert.[7] The growth curves of Group B *Streptococcus* type III (893) in the various amniotic fluid plus zinc solutions are shown in Fig. 1. The bacteria proliferated rapidly in these solutions at rates that equaled or exceeded their growth in amniotic fluid alone. The enhancement of bacterial growth varied with the concentration of zinc in the amniotic fluid: 700 and 70 μmol solutions caused a greater proliferation than the 7.0 μmol solution. The 0.7 μmol zinc solution caused the least profound bacterial growth. The 24-hour growth curves of Group B *Streptococcus* type III (893) in amniotic fluid plus phosphorus are shown in Fig. 2. The presence of phosphorus in the amniotic fluid samples resulted in inhibition of growth. This inhibition varied inversely with the concentrations of phosphorus (the higher concen-

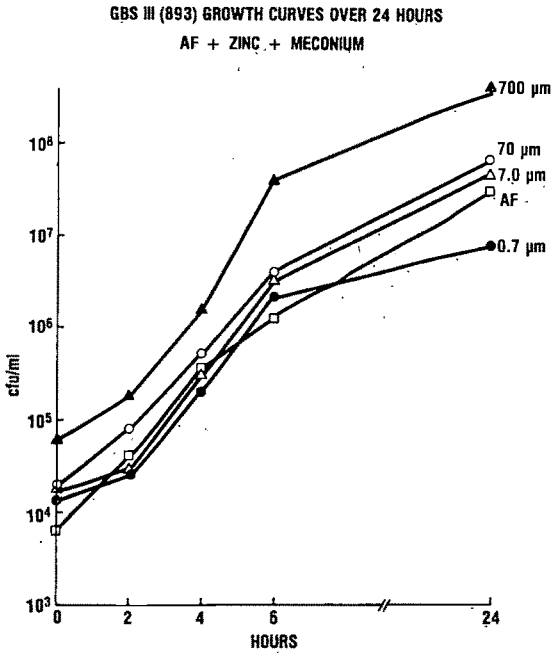


Fig. 3. Proliferation of Group B *Streptococcus* III (893) growth in amniotic fluid that contained zinc in the presence of meconium.

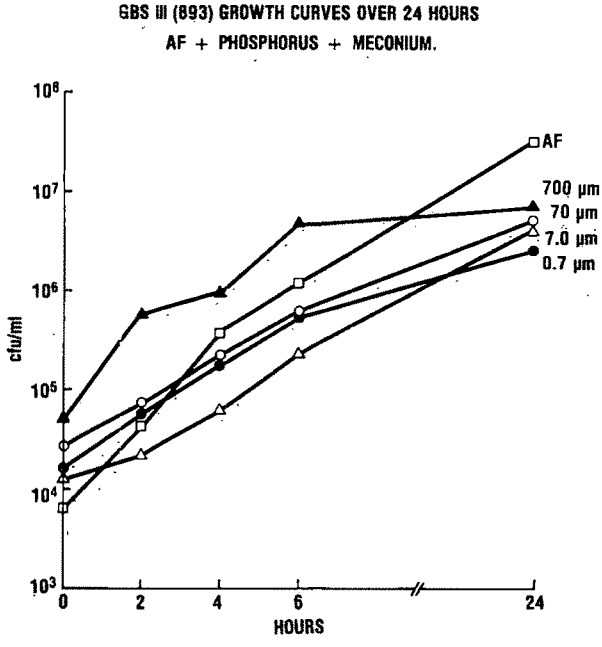


Fig. 4. Group III (893) over 24 hours after the addition of meconium to amniotic fluid that contained phosphorus.

trations were least inhibitory). The zinc and phosphorus contents in meconium were 70 and 128 µg/ml, respectively. The results of the addition of the meconium to the amniotic fluid plus zinc solutions are shown in Fig. 3. The addition of meconium resulted in the modulation of the growth-enhancing effect of the zinc, which thus demonstrated a 1- to 2-log increase in the number of organisms per milliliter after its addition to the amniotic fluid plus zinc solutions. The results of the addition of meconium to the amniotic fluid plus phosphorus solutions are shown in Fig. 4. Meconium blocked the growth-inhibiting effect of phosphorus on Group B *Streptococcus* growth, which showed a 1- to 2-log increase in the organisms, even though the overall growth was less proliferative than in amniotic fluid alone. Although the *E. coli* grew well in the fluid, their growth was inhibited by the addition of the zinc solutions and was enhanced by the addition of phosphorus to the fluid. These results are similar to those reported by Schlievert et al.,[7] who also described an inhibitory effect of zinc on the growth of *E. coli* with strain 06.

Comment

Group B *Streptococci* are important agents that influence perinatal morbidity and mortality. Although the literature describes various factors in amniotic fluid that impede bacterial growth, little is known about the influence of these factors on the prevention of Group B *Streptococcus* growth. Some human amniotic fluid specimens have impeded the in vitro proliferation of certain bacterial strains, whereas others have supported their vigorous growth. In a previous report,[3] we demonstrated the strain-specific variation in Group B *Streptococcus* growth curves in human amniotic fluid in vitro. Group B *Streptococcus* type III strains 893 and 891 proliferated more vigorously than strain 878.

Whether the zinc peptide inhibits Group B *Streptococcus* proliferation in amniotic fluid is not known with certainty. Schlievert et al.[7] isolated a zinc-dependent peptide from human amniotic fluid, which inhibited the proliferation of strains of *E. coli* 06, *S. aureus*, and *S. faecalis*. Further, they demonstrated that ratios of phosphorus to zinc in amniotic fluid may predict the in vivo activity of the peptide against the specific organism tested. A ratio of <100 was bactericidal; a ratio between 100 and 200 was bacteriostatic; and a ratio of >200 was described as noninhibitory. Others sought evidence to support the predictive value of phosphorus-to-zinc ratios with discrepant results. The findings reported in the present study are similar to those of Blanco et al.[2] These authors[2] have also described an inhibition of bacterial growth in the presence of phosphorus in amniotic fluid. Although the theory of Schlievert et al.[7] of the inhibitory effect of a zinc peptide on the growth of some organisms in amniotic fluid may be valid with certain bacteria such as *E. coli* and *S. aureus*, it apparently does not apply to Group B *Streptococcus*. Also, the growth of these organisms in the amniotic fluid plus phosphorus solutions differed from that of *E coli*. With *E. coli* the colony counts after

24 hours in the fluid plus phosphorus solutions exceeded their growth in amniotic fluid alone. The Group B *Streptococcus* colony counts at 24 hours in the amniotic fluid plus phosphorus solutions, however, were consistently 1- to 2-log units below the counts seen in amniotic fluid alone.

The reason for the differences may lie in the growth requirements of the Group B *Streptococcus*. As previously mentioned, Blanco et al.[2] have described the effects of differing phosphate concentrations on various species of streptococci.[2] The absence of phosphate decreases the growth of streptococci, but so does increasing the concentrations of phosphate. These requirements may have a role in the findings described in this study. Compared with the results of the growth curves of *E. coli*, the organisms proliferated with the addition of increasing concentrations of zinc to amniotic fluid, and their growth was inhibited by the addition of increasing concentrations of phosphate to the fluid. We can speculate that the pathogenicity of the Group B *Streptococcus* may vary not only with the specific strain but also with the differences in the levels of and therefore the ratios of zinc and phosphorus in amniotic fluid.

Several investigators[8-11] have demonstrated that amniotic fluid by itself is a poor culture medium for bacteria. However, Florman and Teubner[4] have shown that when relatively small amounts of meconium are added to the fluid, it becomes a good culture medium for bacterial growth. Our findings are similar and demonstrate the growth patterns of Group B *Streptococcus* in amniotic fluid after the addition of meconium. This growth is seen as an enhanced proliferation of the bacteria in the amniotic fluid solutions that contain zinc and a blocking of the inhibition of this growth in the amniotic fluid plus phosphorus solutions.

Meconium is a poorly defined and inconstant substance that contains various mucopolysaccharides and inorganic elements. It is unclear as to which of these elements converts amniotic fluid into a good culture medium for bacteria. One possible mechanism whereby this occurs may be by an alteration of the zinc-to-phosphorus ratios in the fluid, thus resulting in bacterial proliferation. These observations suggest that the fetus who is at risk for developing Group B *Streptococcus* infection from the mother and who remains in an environment that contains meconium may be at increased risk from this potentially lethal disease.

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Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow

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Umbilical artery Doppler blood flow velocity studies were used to identify 14 fetuses with absent flow during diastole to determine the significance of absent umbilical artery diastolic flow. Outcomes of these fetuses were recorded, and the associated intracardiac Doppler changes were identified in 12 of them. Maximal and mean intracardiac flow velocities were measured, and volume flows through the right (tricuspid valve, pulmonary valve) and left (mitral valve, aortic valve) sides of the heart were compared. Ratios of intracardiac peak flow velocity in late diastole to peak flow velocity in early diastole were calculated. Eleven fetuses had intrauterine growth retardation, and four had multiple congenital anomalies. Fetuses with no diastolic flow in the umbilical artery had increased volume flow across the tricuspid and pulmonary valves compared with normal fetuses of similar weights. The ratio of right-sided to left-sided volume flow in the heart (2.15:1) was increased compared with values in normal fetuses (1.33:1, $p < 0.01$). The ratio of late diastolic to early diastolic peak flow velocities across the mitral valve was decreased ($p < 0.01$). Absent umbilical artery diastolic flow is associated with increased tricuspid and pulmonary valve volume flow and changes in mitral flow velocity patterns, which suggests that there are alterations in left ventricular function. (AM J OBSTET GYNECOL 1987;157:774-9.)

Key words: Fetal heart, Doppler

Doppler investigations of the umbilical artery have been reported to identify fetuses at increased risk for poor fetal outcome.[1-4] Normally, flow through the umbilical artery occurs in all phases of the cardiac cycle partly because of the low resistance of the placental circulation (Fig. 1).[1-4] Increased ratios of umbilical artery systolic to diastolic flow detected by Doppler investigation have been associated with increased perinatal morbidity and mortality and intrauterine growth retardation.[1-4] These studies attribute poor fetal outcome to placental vascular changes; however, examinations of the contribution of the fetal heart to the abnormal waveform and poor fetal outcome have not been performed. Placental vasculature in fetuses with intrauterine growth retardation has been reported to have increased resistance.[2-3] This increase in afterload for the fetal heart may result in changes that are detectable in utero.

Studies of intracardiac physiology in the human fetus witht Doppler are now possible.[5-10] Studies in normal fetal hearts indicate that volume flow is increased through the fetal right heart compared with the left heart.[6, 8, 9] Fetal intracardiac diastolic Doppler flow velocity waveforms reveal a high peak during late diastole (after atrial contraction) compared with the peak during early diastole.[7, 8]

The purpose of this study was to examine fetuses with no umbilical artery Doppler blood flow velocity during diastole, an extreme example of umbilical artery Doppler abnormality. We also studied the intracardiac Doppler flow velocities in these fetuses to document the changes in cardiovascular disease associated with absent umbilical artery diastolic flow.

Material and methods

Fetuses were examined with ultrasound for indications of a hypertensive disorder of pregnancy, suspected growth retardation, or suspected fetal anomaly. Permission was obtained from each mother to perform Doppler studies. Approval for this project had been obtained from the Human Subjects Committee.

Standard ultrasound measurements were obtained, including biparietal diameter, femur length, abdominal diameters, and estimated amniotic fluid volume. Placental location was identified, and general fetal anatomy was assessed. The fetal heart was examined in detail, including four-chamber, short-axis/great vessel, long-axis/great vessel, and arch (aortic and pulmonary artery) views. Cardiac valve diameters were measured with two-dimensional images.

Umbilical artery pulsed Doppler waveforms were recorded from at least four regions with 10 beats each.

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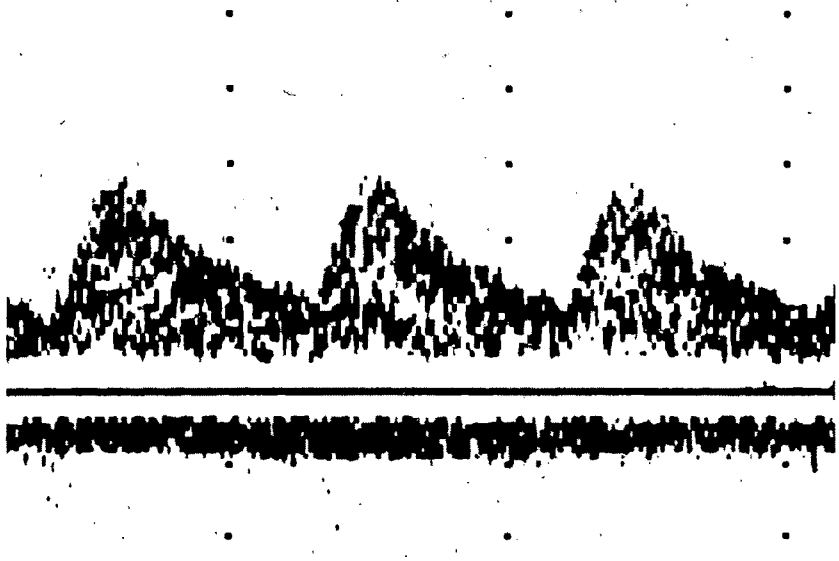


Fig. 1. Doppler flow velocity tracing of the umbilical artery and vein in a normal fetus.

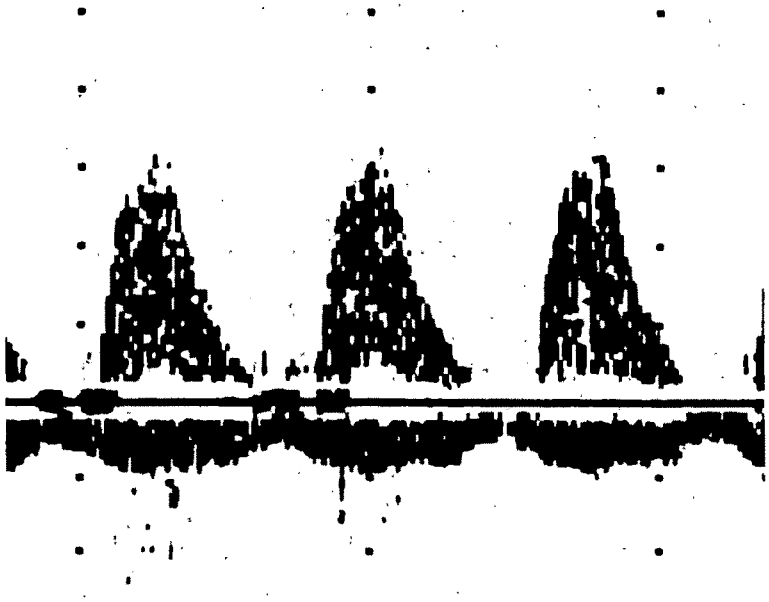


Fig. 2. Doppler flow velocity tracing of the umbilical artery and vein in a fetus with absent diastolic umbilical artery blood flow. Velocity decreases to zero after each peak. Fluctuations in venous flow velocity are not caused by fetal breathing or movement.

Fetuses were included in the study if no umbilical artery flow was present during diastole (Fig. 2).

Pulsed Doppler investigations of the intracardiac chambers and great vessels were performed as previously described.[6] With two-dimensional guidance, the pulsed Doppler gate was placed immediately distal to the valve leaflets of the cardiac region being sampled. The direction of blood flow was estimated to be perpendicular to the valve anulus. Velocity measurements were accepted if the angle of insonation was <30 degrees from the estimated direction of blood flow. Maximal and mean velocity measurements were obtained from page print outputs from a strip chart recorder. Maximal velocity was measured from the zero line to the highest point of the velocity peak. Temporal mean velocity was calculated by planimetering the area beneath the gray scale spectral velocity display and dividing by the time over which the velocity was traced.

Table I. Tricuspid and mitral valve values (mean ± SEM)

	Normal[6,7] *(n = 47)*	*No diastolic flow (n = 12)*	*p*
Tricuspid			
Maximal velocity (cm/sec)	51 ± 4	42 ± 2	<0.001
Mean velocity (cm/sec)	12 ± 1	13 ± 1	<0.05
Diameter (mm)	8.0 ± 0.5	9.5 ± 0.6	NS
Volume flow (ml/kg/min)	307 ± 30	476 ± 15	<0.05
A-to-E ratio	1.29 ± 0.04	1.22 ± 0.04	NS
Mitral			
Maximal velocity (cm/sec)	47 ± 4	34 ± 3	<0.001
Mean velocity (cm/sec)	11 ± 1	10 ± 2	NS
Diameter (mm)	6.6 ± 0.4	7.7 ± 0.4	NS
Volume flow (ml/kg/min)	232 ± 25	232 ± 11	NS
A-to-E ratio	1.35 ± 0.01	1.00 ± 0.04	<0.01

A = Peak flow velocity during late diastole; E = peak flow velocity during early diastole; NS = not significant.

Table II. Pulmonary and aortic valve values (mean ± SEM)

	Normal[6,9] *(n = 32)*	*No diastolic flow (n = 10)*	*p*
Pulmonary			
Maximal velocity (cm/sec)	60 ± 4	56 ± 2	NS
Mean velocity (cm/sec)	16 ± 2	18 ± 2	<0.01
Diameter (mm)	7.6 ± 0.3	8.0 ± 0.3	NS
Volume flow (ml/kg/min)	312 ± 11	450 ± 11	<0.01
Aorta			
Maximal velocity (cm/sec)	70 ± 3	55 ± 3	<0.001
Mean velocity (cm/sec)	18 ± 2	13 ± 2	<0.001
Diameter (mm)	6.7 ± 0.2	6.6 ± 0.2	NS
Volume flow (ml/kg/min)	250 ± 9	222 ± 9	NS

Volume flow was calculated by multiplying mean velocity by the area (πr^2), through which blood was flowing. Neonatal weights were used for calculating volume flow.

Two-dimensional and Doppler measurements obtained from patients in the study were compared with those of normal fetuses of similar weights, which have been reported elsewhere.[6, 7, 9] Values reported are mean ± SEM, except where otherwise noted.

The instrument used was a 3.5 MHz scanner with pulsed Doppler capability. According to the manufacturer's specifications, fetuses were exposed to <46 mW/cm^2 ultrasound power density. Pregnancies were managed without relying on Doppler information. The outcomes of the fetuses were recorded, including method and reason for delivery, weight, Apgar scores, admission to the intensive care unit, and presence of congenital anomalies. The statistical method used was Student's *t* test for unpaired values, with significance at 0.05.

Results

Fourteen fetuses were included in this series, with intracardiac studies in 12 fetuses. Maternal age was 24.6 ± 5.2 (mean ± SD) years, gravidity was 2.4 ± 1.7, and parity was 0.9 ± 1.1. Hypertensive disorders complicated 71% of the pregnancies, and fetal anomalies were suspected in 29%. Amniotic fluid volume was decreased or absent in 57%.

Vaginal deliveries occurred in 43% of the patients; in only one case was this a vertex vaginal delivery of a live infant and consisted of midforceps for fetal distress. Cesarean sections were performed in eight of 14 (57%), in each case for fetal distress, defined by the presence of late decelerations detected with electronic fetal monitoring. Four (29%) fetuses died in utero, three with known anomalies (trisomy 13, suspected trisomy 13, and trisomy 21); one mother of an otherwise normal fetus refused cesarean section with late decelerations with electronic fetal monitoring. Pericardial or pleural effusions were present in four fetuses. In total, four (29%) of the fetuses had multiple congenital anomalies, including the trisomic fetuses, and one had hypoplastic lungs and polycystic kidneys. Two neonates died, one of multiple congenital anomalies and one of unknown causes. All live fetuses were admitted to the neonatal intensive care unit. Eleven (78%) were growth-retarded by institutional standards. Two of three fetuses without growth retardation were anomalous (one with cystic hygromas and trisomy 21 and one with polycystic kidneys). The total perinatal mortality was 42%.

The mean fetal biparietal diameter was 6.9 ± 1.2 cm

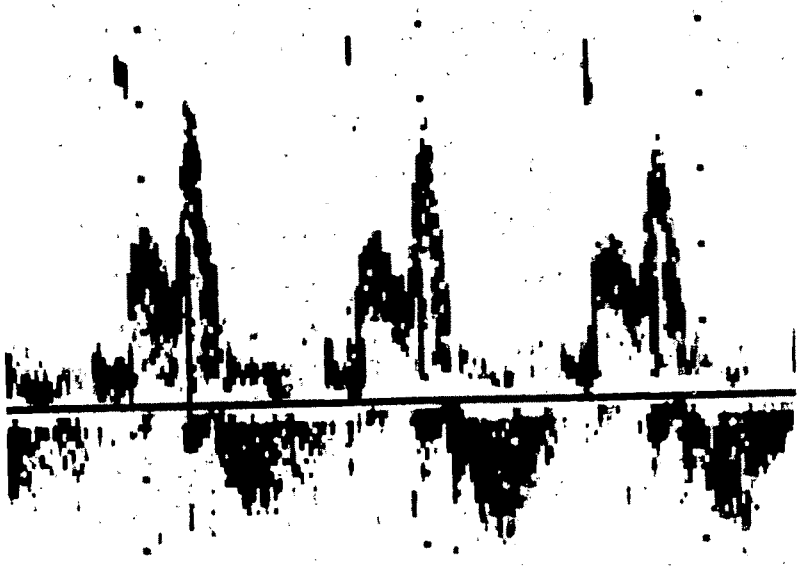


Fig. 3. Doppler flow velocity tracing across the mitral valve in a normal fetus. Two peaks are normally present during diastolic flow, one in early diastole (E) and one in later diastole (A). In the normal human fetus, the A is usually higher than the E, resulting in an A-to-E ratio of >1.

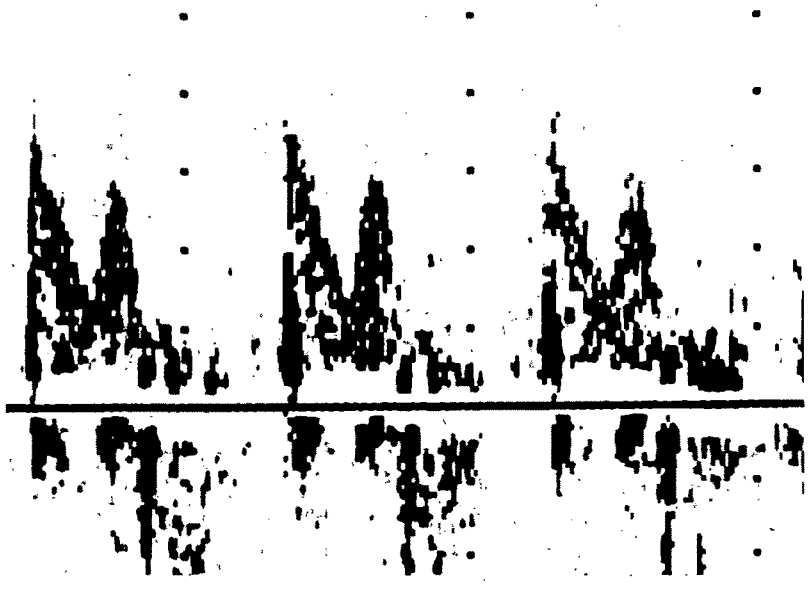


Fig. 4. Doppler flow velocity tracing across the mitral valve in a fetus with absent umbilical artery diastolic flow. A-to-E ratio is <1.

(mean ± SD), mean fetal femur length was 5.2 ± 1.0 cm, and mean estimated gestational age at diagnosis was 32 ± 5 weeks. Deliveries or fetal death occurred an average of 6 days after diagnosis (range, 0 to 27 days). Mean neonatal weight was 1227 ± 255 gm, with a 1-minute Apgar score of 5 ± 3 and a 5-minute Apgar score of 7 ± 3.

The two-dimensional ultrasound and Doppler results are shown in Tables I and II. Tricuspid and pulmonary volume flows were increased compared with normal values. The ratio of peak flow velocity during late diastole to peak flow velocity during early diastole was decreased (Table I, Figs. 3 and 4). Tricuspid valve measurements (maximal velocity, $p < 0.05$; mean

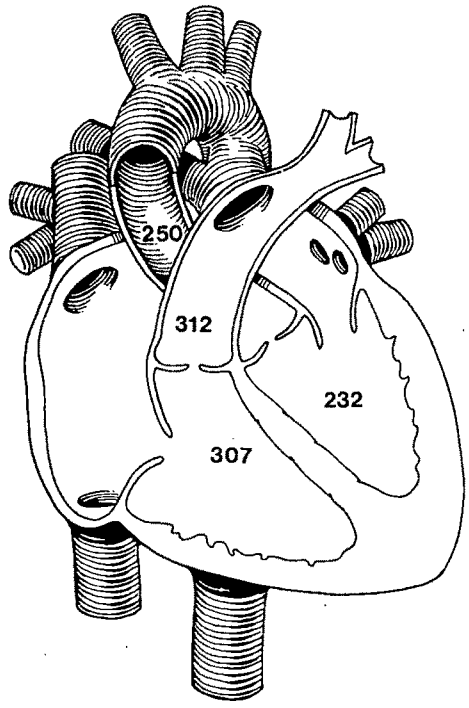


Fig. 5. Volume flow in the normal fetal heart. Numbers represent milliliters per kilogram per minute. *307* = Tricuspid valve, *312* = pulmonary valve, *232* = mitral valve, and *250* = aortic valve.

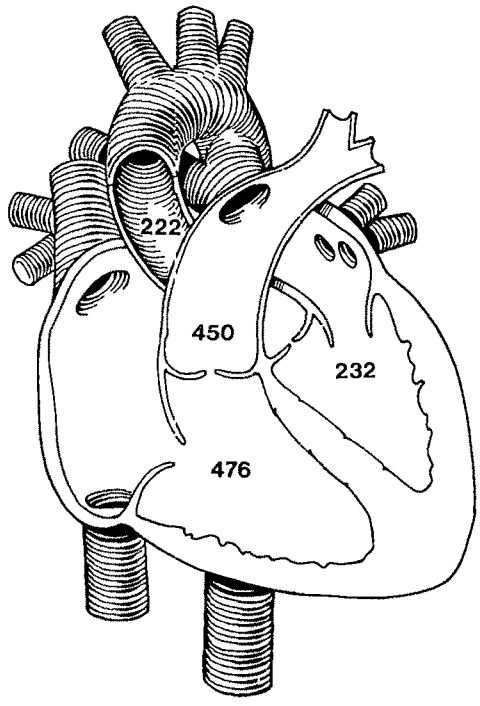


Fig. 6. Volume flow in the heart of a fetus with absent diastolic flow in the umbilical artery. Numbers represent milliliters per kilogram per minute. *476* = Tricuspid valve, *450* = pulmonary valve, *232* = mitral valve, and *222* = aortic valve.

velocity, $p < 0.01$; diameter, $p < 0.05$; and volume flow, $p < 0.05$) were greater than those of the mitral valve in the abnormal group. Pulmonary valve measurements (mean velocity, $p < 0.001$; diameter, $p < 0.01$; and volume flow, $p < 0.001$) were greater than those of the aortic valve in the abnormal group. The ratio of tricuspid valve to mitral valve volume flow was increased ($2.32 \pm 0.03:1$) compared with normal ($1.37 \pm 0.05:1$, $p < 0.01$). The ratio of pulmonary valve to aortic valve flow was also increased ($2.15 \pm 0.03:1$) compared with normal ($1.33 \pm 0.02:1$, $p < 0.001$, Figs. 5 and 6). In individual fetuses no significant differences were found when tricuspid valve volume flows were compared with pulmonary valve volume flows (right-sided flows) and mitral valve volume flows were compared with aortic valve volume flows (left-sided flows).

Comment

Changes in the pattern of blood flow velocity in the umbilical artery are thought to reflect placental function and fetal well-being.[1-4] In the normal fetus, the ratio of systolic to diastolic flow decreases with advancing gestational age. If this trend is reversed and the ratio is increased, an abnormality of the fetoplacental vasculature may be present. An extreme example of this is the umbilical artery Doppler flow velocity waveform without flow during diastole, which is therefore highly abnormal.

In this report, umbilical artery Doppler flow velocity studies were used to identify fetuses with absent blood flow velocity during diastole. Perinatal morbidity (100%) and mortality (42%) were markedly abnormal in this group. Intracardiac Doppler studies were performed in these fetuses to examine the physiologic changes in the fetal heart that accompany these abnormal umbilical flow velocity patterns. Volume flows were increased on the right side of the fetal heart (tricuspid and pulmonary artery valves) compared with the left side of the heart (mitral and aortic valves) and compared with normal fetuses of similar weights.

Previous studies of intracardiac volume flow in normal fetuses have shown that the fetal right heart volume flows are greater than left heart volume flows by a ratio of $1.3:1$.[6, 8] In this study we found an even greater increase in the right heart flow, with a tricuspid valve–to–mitral valve volume flow ratio of $2.32:1$ and a pulmonary valve–to–aortic valve volume flow ratio of $2.15:1$. Left heart volume flow was unchanged compared with normal. In addition, a decrease in the ratio of late diastolic peak flow velocity to early diastolic peak flow velocity in the mitral valve suggested that there may also be a change in the diastolic function in the fetal left ventricle.

Our findings regarding fetal outcome are consistent with previous investigators who showed almost identical data (cesarean section for fetal distress, 75%; perinatal mortality, 40%[11]) in fetuses with no flow in the umbilical artery during diastole. Our report is the first investigation to our knowledge that attempts to examine the pathologic mechanism that may be responsible for these outcomes.

One possible explanation for the intracardiac changes we report is that developing hypoxia results in dilation of the cerebral vasculature, as evidenced indirectly by fetal cerebral artery studies. These studies indicate that a decrease in the ratio of systolic to diastolic measurements in the cerebral arteries is associated with the development of fetal growth retardation.[12] In addition, in hypoxic neonates, the pulmonary vasculature constricts while the ductus enlarges. To increase volume blood flow to the brain from the left ventricle, in the face of a dilated ductus supplied by the right ventricle, volume flow must increase across both ventricles. This interpretation is consistent with the findings of our study, with increased volume flow through the right side of the heart and increased combined ventricular output.

The alteration in flow across the mitral valve with a shift to more flow in early diastole is interesting. Previous investigators have shown that the normal fetal ventricle is noncompliant[13]; Doppler investigations have shown that the fetal diastolic function changes with advancing gestational age in a pattern consistent with gradually increasing compliance.[7] The changes in mitral flow velocity waveforms seen in this study of abnormal fetuses may be based on a relative increase in left ventricular volume, which results in dilation and increased compliance. Increases in right and left ventricular diameters have been reported in the presence of fetal growth failure.[14]

We conclude from this study that the presence of umbilical artery blood flow velocity waveforms with no flow during diastole is associated with an increase in perinatal morbidity and mortality. Alterations in intracardiac Doppler flow velocity waveforms are detectable in these fetuses. These changes may explain the pathophysiology in the cardiovascular system that accompanies fetal growth failure and fetal distress.

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Enhancement of maternal and neonatal natural killer cell activity with interleukin-2

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Natural killer cell activity, which is vital to the immune surveillance against viruses and tumor cells, is significantly lower in the newborn infant than in the mother. Incubation of maternal and cord blood lymphocytes with interleukin-2 significantly increases natural killer activity in both. The increase is substantially greater in the cord blood lymphocytes. (AM J OBSTET GYNECOL 1987;157:780-1.)

Key words: Natural killer cell activity, cord blood, interleukin-2

Natural killer cells are reported to be important in immune surveillance destruction of viruses and tumor cells.[1] Studies have shown that newborn infants have low natural killer activity. This study was undertaken to establish whether interleukin-2, a known immunomodulator,[2] can enhance natural killer cell activity in maternal and cord blood lymphocytes.

Material and methods

Fifteen pairs of samples of maternal and cord blood were obtained at the time of delivery. All deliveries were singleton, at term, and without complications. The protocol was previously approved by the Investigational Review Board. Lymphocytes were separated by Ficoll-Hypaque density centrifugation, washed with and resuspended in RPMI-1640, which contained 10% fetal calf serum. Natural killer cell activity was determined with a 4-hour chromium 51 release assay with K 562 cells as targets. Overnight incubation of lymphocytes, with and without interleukin-2, was at 37° C in the presence of 5% carbon dioxide. All assays were performed at four effector to target ratios (100, 50, 25, and 12.5:1). An aliquot of the lymphocytes obtained in four of the 15 sets of blood samples was labeled with the Becton-Dickinson monoclonal antibodies Leu 7 and Leu 11 that identify natural killer cells.

A total of 10,000 cells was counted in each determination of Leu 7^+ and Leu 11^+ cells with a fluorescence-activated cell sorter. Statistical analyses were done with Student's paired *t* test.

Results and comment

As shown in Table I, cord blood natural killer activity is significantly lower than that of the maternal blood

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Table I. Effect of interleukin-2 on natural killer activity and percent of Leu 7^+ and Leu 11^+ cells in maternal and cord blood samples (effector to target ratio, 50:1)

	Day 0	*Day 1*	*Day 1 + 20 U/ml interleukin-2*		*Day 1 + 80 U/ml interleukin-2*
Natural killer activity (mean ± SEM)					
Maternal (n = 15)	25.4 ± 4.7	18.0 ± 2.3	$p < 0.0003$	39.3 ± 5.7	43.1 ± 4.7
	$p < 0.04$				
Cord (n = 15)	14.0 ± 2.2	15.7 ± 3.5	$p < 0.0$	30.2 ± 5.4	48.6 ± 6.9
Increase (%) (mean ± SEM)					
Maternal (n = 15)				211.4 ± 11.2	256.8 ± 17.9
				$p < 0.05$	$p < 0.04$
Cord (n = 15)				306.5 ± 41.6	374.7 ± 46.7
Leu 7^+					
Maternal (n = 4)	11.6 ± 3.0	9.3 ± 2.5		11.8 ± 3.0	13.0 ± 2.0
Cord (n = 4)	4.4 ± 1.8	4.2 ± 1.9		5.2 ± 2.1	4.9 ± 1.8
Leu 11^+					
Maternal (n = 4)	17.6 ± 3.9	17.6 ± 3.5		19.4 ± 3.8	18.5 ± 3.0
Cord (n = 4)	17.4 ± 2.9	19.5 ± 2.8		20.7 ± 3.1	20.5 ± 30

on the day of delivery (day 0). Overnight incubation with 20 U/ml interleukin-2 shows a significant increase in natural killer activity in both sets, compared with the lymphocytes incubated alone (day 1). However, the increase in the cord blood natural killer activity is significantly greater than that of the maternal blood. Incubation with 80 U/ml interleukin-2 showed further increases in natural killer activity, but they were not significantly different when compared with incubation with 20 U/ml interleukin-2. Table I presents the natural killer activity data at a 50:1 effector to target ratio. At the other effector to target ratios of 100:1, 25:1, and 12.5:1, the findings were identical. Leu 11^+ cells were identical in the two groups, but there was a decrease in Leu 7^+ cells in cord blood. Interleukin-2 treatment resulted in no changes. The data suggest that natural killer activity in the newborn infant requires maturation and that this process can be enhanced by interleukin-2. Additional investigations with animal models with specific viral infections (herpesvirus and cytomegalovirus) could define these relationships of natural killer cells, their function in viral infection, and their potentiation with lymphokines. Interleukin-2 might have efficacy in the newborn infant's antiviral therapy; however, this can be proved only with careful detailed studies in animals and in vitro.

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Maternal serum α-fetoprotein screening: Effects of weight adjustment

To the Editors:

In the August 1986 issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, Dr. Macri (Macri JN. Critical issues in prenatal maternal serum α-fetoprotein screening for genetic anomalies. AM J OBSTET GYNECOL 1986;155:240) suggested that adjustment of the maternal serum α-fetoprotein for maternal weight would have a negative effect on the screening process. He based his argument largely on the data of Wald et al.[1] published in 1981, which showed that in pregnancies in which the fetus is affected with open spina bifida, the maternal weight was found to be approximately 1.6 kg less than that in unaffected pregnancies. Dr. Macri further stated that had weight adjustment been incorporated into the interpretations of maternal serum α-fetoprotein results in his screening population, "a significant reduction of NTD detection efficiency would have occurred." Both Dr. Wald and Dr. Macri are concerned with the relatively small woman whose maternal serum α-fetoprotein level may be only slightly elevated and in whom adjustment for maternal weight will move the value into the normal range.

Neither author addressed the issue of weight adjustment in the large woman in whom a neural tube defect might be missed because of an unadjusted value that falls within the normal range; nor did they address the issue of weight adjustment at the lower end of the scale, where weight adjustment may affect the decision to offer amniocentesis for detection of chromosomal abnormalities.

Other investigators have demonstrated that adjustment for maternal weight can increase the specificity of the test with no loss in sensitivity[2] and that weight correction is especially important if α-fetoprotein levels are to be used to predict the risk of neural tube defects or trisomy 21.[3]

Our experience in Maryland in screening 7116 women supports the rationale of weight adjustment. We multiply the multiple of the median value by the ratio of the patient's weight to 135 pounds. The following case studies illustrate the utility of this adjustment.

In Case 1, a woman weighing 214 pounds had a maternal serum α-fetoprotein result of 60.1 ng/ml or 1.6 multiples of the median for 17 weeks' gestation. No further workup was indicated with this interpretation. However, after correction for the maternal weight, the maternal serum α-fetoprotein value became 2.5 multiples of the median and a repeat sample was requested.

The second maternal serum α-fetoprotein value, determined at 18 weeks' gestation, was 75.1 ng/ml or 1.9 multiples of the median, corrected for weight to 3.0 multiples of the median.

Level II ultrasound showed fetal meningomyelocele, hydrocephalus, and scoliosis.

The amniotic fluid α-fetoprotein was 4.0 multiples of the median and the acetylcholinesterase test results were strongly positive. Chromosomes were normal.

In Case 2, a 31-year-old woman weighing 115 pounds had a maternal serum α-fetoprotein result of 11 ng/ml or 0.4 multiple of the median for 16 weeks. The lower cutoff of the laboratory at this time was 0.3 multiple of the median (regardless of age). With correction for the maternal weight of 115 pounds the maternal serum α-fetoprotein multiple of the median decreased to 0.3 and amniocentesis to rule out Down syndrome was offered. A diagnosis of trisomy 21 was made by chromosomal analysis.

The Maryland State Health Department α-Fetoprotein Laboratory, which incorporated weight adjustment into its maternal serum α-fetoprotein interpretations, has not missed any neural tube defects because of a reduction of the maternal serum α-fetoprotein values in the group of women whose borderline elevated values were adjusted into the normal range because of maternal weight. We have, on the contrary, avoided many unnecessary amniocenteses in these patients. More importantly, we have diagnosed one case of open spina bifida and one case of trisomy 21 that might otherwise have been missed.

We would be interested in specific figures from Dr. Macri's screening program to illustrate his point concerning a negative effect on detection in the group of "small women." We would also be interested in data from other screening programs related to their experience with adjustment of the maternal serum α-fetoprotein value for maternal weight.

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Reply

To the Editors:

We are pleased to respond to the comments of Ms. Kazazian, Dr. Niebyl, and Dr. Joseph on maternal weight data presented in our August 1986 article.

Some concerns raised by these authors were addressed by us in a subsequent article in October 1986 entitled, "Maternal serum α-fetoprotein screening, maternal weight, and detection efficiency."[1] A review of this more recent communication from our laboratory would have removed their concerns that we based our argument largely on data from others, that we are concerned with relatively small women, and that we did not address the issue of weight adjustment in large women. Data presented in our October 1986 article demonstrates that our concerns about maternal weight adjustment stem from our analysis of a sizable pregnancy population and the effect of weight adjustment by three published methods. Applied to our data, all three methods result in a larger proportion and a different group of women being alerted to the need for further evaluation, and in two of the three methods detection efficiency* is negatively influenced.

Kazazian et al. refer to Palomaki et al.[2] and state that: "Other investigators have *demonstrated* (italics added) that adjustment for maternal weight can increase the specificity of the test with no loss in sensitivity. . . ." Analysis of data from Palomaki et al. reveals that conclusions drawn in their article are based on assumptions and estimates and not on achieved detection efficiency. In fact, it is unclear to us why Palomaki et al. chose to base their conclusions on assumptions rather than use actual patient data and pregnancy outcome. In the 2 years since publication of the article by Palomaki et al., empirical data and pregnancy outcome information to support their theory have not appeared from them or others. We are unclear therefore as to how Kazazian et al. can claim the "demonstration" of the effectiveness of maternal weight adjustment.

Kazazian et al. indicate that their screening experience in Maryland supports the rationale of weight adjustment. In fact, the method of weight adjustment suggested by the Johns Hopkins group (multiplying the actual multiple of the median value by the ratio of maternal weight to 135 pounds) is quite different from published methods suggested by other investigators (the absolute α-fetoprotein value is divided by an expected α-fetoprotein value to determine an adjusted multiple of the median). The Johns Hopkins group, however, do not present data concerning the scientific basis for or the specific effect of their new approach. We have therefore applied their approach to the same pregnancy population evaluated in our October 1986 article in which three published weight adjustment methods were assessed. The Johns Hopkins group method resulted in the reclassification of (1) 65 patients as having normal levels and (2) 147 patients as having elevated levels. This method demonstrates an even more unfavorable net effect of weight adjustment as it relates to reclassification of maternal serum α-fetoprotein levels into the elevated category than those three methods evaluated in our October 1986 article. Inasmuch as the Johns Hopkins group method has not been published and since no explanation was provided by Kazazian et al., readers will be confused as to whether a decision not to reclassify patients into the elevated category (as recommended by Wald et al.[3]) is followed by these authors. Irrespective of the false positive issue, the Johns Hopkins group method also results in a loss in detection efficiency (one of four cases of open spina bifida reclassified as having a normal level).

To support the beneficial aspects of their weight adjustment approach, Kazazian et al. presented two case reports. Careful analysis of these case reports reveals some specific concerns. First, it is clear that the median maternal serum α-fetoprotein values at 16, 17, and 18 weeks are 27.5, 37.6, and 39.5 ng/ml, respectively. This represents an unusual increase in median levels between 16 and 17 weeks (36.7%) and only a small increase between 17 and 18 weeks (5.05%). These differences are contrary to what is known of weekly increases in maternal serum α-fetoprotein. Indeed material from the Maryland maternal serum α-fetoprotein screening program indicates that in their data base, normative data for weeks 20 to 25 were estimated on the basis of the assumption that α-fetoprotein increases by 15% per week. The above Maryland data for 16 to 18 weeks' gestation do not support this assumption. The effect of such unusual maternal serum α-fetoprotein normative data on the percentage of elevated samples, as well as on detection efficiency, causes concern. This concern is heightened when we consider the following statements from one α-fetoprotein quality assessment scheme[4] (in which the Maryland maternal serum α-fetoprotein program participates): "In most cases the problems with interpretation arise from inaccurate reference data (medians). Examination of assay results in conjunction with supplied medians, reveals that in some cases the problems lie with inappropriate medians, either much too low or too high. In addition, medians in some cases are inconsistent, i.e. do not increase by the expected 12% to 18% per week, with the result that multiple of the median calculations are highly erratic depending on the gestational age of the specimen."

Second, Kazazian et al. use a cutoff of 0.3 multiple of the median (regardless of age) when screening for Down syndrome. Although screening for Down syndrome is in an investigative stage, a 0.3 multiple of the median cutoff does not seem adequate. It should be noted that preliminary data indicate that the median α-fetoprotein level for Down syndrome cases is 0.7 mul-

*Data presented in our article pertain to maternal serum α-fetoprotein screening for structural malformations. Effect of weight adjustment on Down syndrome screening is under investigation.

tiple of the median; so the use of a 0.3 multiple of the median cutoff could potentially lead to poor detection efficiency. Additionally, since the incidence of Down syndrome increases substantially with age, failure to incorporate age in determining risk will result in misinterpretation of low values of α-fetoprotein.*

With regard to Kazazian et al.'s Case 2, the patient's weight-adjusted multiple of the median is 0.34. Inasmuch as 0.34 multiple of the median is higher than the authors' 0.3 multiple of the median cutoff, we are unclear as to why their laboratory alerted clinicians to the need for further evaluation in this case.

Kazazian et al. concluded by stating that the Maryland State Health Department has not missed an open neural tube defect because of maternal weight adjustment and, on the contrary, has avoided many unnecessary amniocenteses. They did not reveal, however, (1) the mathematical basis for their weight adjustment system, (2) the number of cases on which it is based, (3) how many pregnancies either (a) avoided further testing or (b) were directed to go on for further testing because of weight adjustment, or (4) their overall program detection efficiency for open neural tube defects. All information concerning weight adjustments published by others to date presents a concept that is based on estimates and assumptions but fails to present empirical data to demonstrate the beneficial effects of this statistical adjustment.

The data presented in our October 1986 maternal weight article illustrate our point concerning a negative effect on detection efficiency. Finally, we share with Kazazian et al. an interest in empirical data from other screening programs related to their experience with weight adjustment of maternal serum α-fetoprotein values.

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*We disagree with the use of the multiple of the median statistical parameter for reporting and decision making. Patient-specific risk reporting is clinically preferable.[5,6]

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Drug prescribing for thromboembolic disease during pregnancy

To the Editors:

Drs. Rayburn and Lavin have done obstetricians a great service in summarizing the current treatment of common disorders complicating pregnancy (Rayburn WF, Lavin JP Jr. Drug prescribing for chronic medical disorders during pregnancy: an overview. Am J Obstet Gynecol 1986;155:565-9). Their protocol for treating thromboembolic disease assumes physicians have a reliable heparin assay available. Unfortunately, most hospitals do not offer tests of heparin levels although they do provide reliable activated partial thromboplastin time determinations.

We have a simplified approach to the treatment of thromboembolic diseases such as deep venous thrombosis or pulmonary embolism. Our regimen, modified from the protocol of Hull et al.,[1] utilizes the activated partial thromboplastin time. The initial treatment in the hospital with intravenous heparin is the same; however, after at least 7 days of therapy patients are switched to heparin administered subcutaneously every 12 hours. The initial daily subcutaneous dose used is two thirds of the total intravenous dose required during 24 hours to maintain a therapeutic activated partial thromboplastin time. The daily subcutaneous dose is divided into two doses given every 12 hours. Thus, if a patient requires intravenous heparin at 1000 units/hr, the total daily intravenous dose is 24,000 units. The patient receives two thirds of this dose (16,000 units) divided into 8000 units subcutaneously every 12 hours. The activated partial thromboplastin time is measured 6 hours after the subcutaneous heparin dose, and the heparin dose is adjusted until a therapeutic activated partial thromboplastin time is achieved (one and a half to two times control). This regimen has proved satisfactory in our hospital in more than 50 cases of deep vein thrombosis. Physicians who do not have a heparin assay available at their hospitals may find our approach acceptable.

The treatment of pregnant patients with prosthetic heart valves who require anticoagulation is much more controversial. A recent consensus conference recommended that these patients be treated during pregnancy with subcutaneous heparin every 12 hours in a dose that prolongs the activated partial thromboplastin time to the therapeutic range (one and a half to two times control) 6 hours after the injection.[2] It is recognized that adequate clinic studies to support this recommendation are lacking.

There are not adequate data to be certain that subcutaneous heparin is the optimal regimen. Oakley and Doherty[3] reported a case of thrombosis of a Hammersmith-Alvarez prosthetic mitral valve in a patient treated with "full anticoagulant doses of heparin subcutaneously."

Despite the risk that heparin will fail to prevent some thromboembolic complications, the alternative, coumarin, is unacceptable. In their classic review, Hall et al.[4] showed that pregnancies exposed to coumarin derivatives had the following outcomes: abnormal live birth, 1/6; abortion or stillbirths; 1/6; and apparently normal births; 2/3.

Iturbe-Alessio et al.[5] recently reported an even higher incidence of coumarin embryopathy: 25% (two of eight) in pregnancies exposed from conception to the seventh to eleventh week and 29.6% (eight of 27) in pregnancies treated with acenocoumarol during all three trimesters.

Other anticoagulant regimens that are ineffective or complicated by hemorrhage include minidose heparin and continuous subcutaneous heparin infusions. Minidose heparin (5000 units subcutaneously every 12 hours) has no place in the prevention of thromboembolism in pregnant patients with prosthetic heart valves requiring anticoagulation. Four of 10 patients treated by Chen et al.[6] had thromboembolism during pregnancy while being treated with minidose heparin. The use of continuous subcutaneous heparin infusion for anticoagulation during pregnancy was associated with major bleeding complications requiring transfusion in four of six patients treated.[7] These regimens should be abandoned.

There is a need to collect information on the outcome of pregnancy in patients with prosthetic heart valves requiring anticoagulation, who are treated with adjusted-dose subcutaneous heparin.

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Lymphocyte-reactive antibodies and recurrent early pregnancy failure

To the Editors:

It was with interest that we read the latest report by Unander and Lindholm on the use of leukocyte-rich erythrocyte concentrate transfusions in women with recurrent early pregnancy failure (Unander AM, Lindholm A. Transfusions of leukocyte-rich erythrocyte concentrates: a successful treatment in selected cases of habitual abortion. Am J Obstet Gynecol 1986;154: 516-20). Recognizing the risks associated with transfusion for viral transfer (human immunovirus, hepatitis, and cytomegalovirus)[1] as well as detrimental sensitization (blood groups, platelet and classical histocompatibility or HLA antigens), these authors retrospectively identified some individuals with recurrent unexplained abortions who would not benefit from leukocyte transfusions. Although limited variables were reported, it was concluded that women without "blocking" antibodies are the women who benefit from third-party transfusions. Because Unander and Lindholm's study was not randomized, they failed to acknowledge the 20% to 60% of subjects that may have been able to carry a pregnancy to term without transfusion treatment. Antipaternal cytotoxic antibodies were not studied as a criterion of those who would not benefit from transfusions, as only 20% to 50% of multiparous women have such antibodies. In contrast, the presence of antipaternal cytotoxic antibodies was a major exclusion criterion used by Mowbray et al.[2] in their "controlled trial" of women with recurrent spontaneous abortion immunized with paternal cells.

In trying to clarify those subjects who may not require immunotherapy, data concerning the expected frequency of blocking antibodies in women with primary and secondary abortion would have been elucidating. After the report by Mowbray et al., we set out to define an expected frequency for the presence of cytotoxic antibodies in women who are recurrent spontaneous aborters. We chose to study nulliparous women with (1) three or more elective abortions and (2) three or more spontaneous abortions, without a viable pregnancy progressing beyond 12 weeks in both groups.

Venous blood was obtained from these women at 4 to 12 weeks after their last elective abortion. Serum lymphocytotoxic antibody was assessed at room temperature by complement-dependent lysis against a panel of 30 individuals' peripheral blood lymphocytes in a 1½-hour two-stage technique at 22° C.[3] Only one of 23 (4%) "recurrent elective aborters" exhibited lymphocytotoxic antibodies. For comparison, the incidence of antibody observed in the sample of primary recurrent spontaneous aborters was 8/63 (13%).

We recognize that our control population may not

be ideal because of the lack of monogamous relationships in recurrent elective aborters and the use of a panel of lymphocytes encompassing the major HLA-A and HLA-B specificities rather than "paternal" lymphocytes. However, we believe that the use of multigravid nulliparous women as a reference control is important for studies concerning women with unexplained recurrent spontaneous abortions. Our results suggest that primary aborters with antipaternal cytotoxic antibodies may in fact represent an infrequent but normal response to a first-trimester pregnancy. Albeit not tested, these women may not benefit from lymphocyte transfusions, similar to the situation in women with "blocking" antibodies.

It is regrettable that further randomized controlled studies of recurrent spontaneous aborters with and without leukocyte transfusions appear to be passé. Without a prospective control group, it will be difficult to identify those women who do not need leukocyte transfusion and its associated risks.

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Basic Sciences for Obstetrics and Gynaecology. Second Edition. Tim Chard and Richard Lilford. 195 pages, illustrated. Berlin, 1986, Springer-Verlag.

Carnitine—Its Role in Lung and Heart Disorders. Satellite Symposium on the Occasion of the Central European Congress for Anesthesiology, Graz, September, 1985. E. Kaiser and A. Lohninger. 140 pages, illustrated. Basel, 1987, S. Karger. $37.25.

Chlamydial Infections. Peter Reeve. 126 pages, illustrated. Berlin, 1987, Springer-Verlag. $35.00.

Clinical Obstetrics. Carl J. Pauerstein. 1017 pages, illustrated. New York, 1987, John Wiley & Sons, Inc. No price listed.

The Computer in Obstetrics and Gynaecology. Edited by Kevin J. Dalton and Rupert Fawdry. 234 pages, illustrated. Oxford, 1987, IRL Press Ltd. $54.00.

Dubois' Lupus Erythematosus. Third Edition. Daniel J. Wallace and Edmund L. Dubois. 775 pages, illustrated. Philadelphia, 1987, Lea & Febiger. $98.50.

Evaluation and Installation of Surgical Laser Systems. Edited by David B. Apfelberg. 324 pages, illustrated. New York, 1987, Springer-Verlag. $69.50.

Female Breast Examination. Claude Annonier. 228 pages, illustrated. Berlin, 1986, Springer-Verlag. $52.00.

Gynecologic Endocrinology. Fourth Edition. Edited by Jay J. Gold and John B. Josimovich. 692 pages, illustrated. New York, 1987, Plenum Publishing Corporation. $85.00.

Healthy High-Fiber Cooking. Jeanette P. Egan. 160 pages, illustrated. Tucson, 1987, HPBooks, Inc. $9.95 (soft cover).

Immunoregulation and Fetal Survival. Edited by Thomas J. Gill III and Thomas G. Wegmann with Eric Nisbet-Brown. 325 pages, illustrated. New York, 1987, Oxford University Press. $49.95.

Manual of Obstetrics: Diagnosis and Therapy. Third Edition. Edited by Kenneth R. Niswander. 475 pages, illustrated. Boston, 1987, Little, Brown & Company. $19.50 (soft cover).

The New Pediatric Guide to Drugs & Vitamins. Edward R. Brace and Kenneth N. Anderson. 288 pages. Tucson, Arizona, 1987, The Body Press. $7.95 (soft cover).

Nutrition in Nursing—The New Approach. Betty Kamen, Ph.D., and E. Lynn Fraley, R.N., Dr.P.H. 216 pages. New Canaan, Connecticut, 1987, Keats Publishing, Inc. $14.95 (soft cover).

Obstetrics, Gynecology, and Infant Mortality. Wrynn Smith, Ph.D. 146 pages, illustrated. New York, 1987, Facts On File Publications. $35.00.

Perinatal Care Delivery Systems: Description and Evaluation in European Community Countries. Edited by Monique Kaminski, Gérard Bréart, Pierre Buekens, Henk J. Huisjes, Gillian McIlwaine, and Hans-Konrad Selbmann. 308 pages. New York, 1986, Oxford University Press. $59.00.

Pregnancy Loss: Medical Therapeutics and Practical Considerations. Edited by James R. Woods, Jr., and Jenifer L. Esposito. 355 pages, illustrated. Baltimore, 1987, Williams & Wilkins. $28.50 (soft cover).

Prenatal Screening, Policies and Values: The Example of Neural Tube Defects. Elena O. Nightingale and Susan B. Meiser. 93 pages. Boston, 1987, Harvard University Press. $10.95 (soft cover).

Recent Advances in Obstetrics and Gynaecology—15. Edited by John Bonnar. 282 pages. New York, 1987, Churchill Livingstone, Inc. $37.00.

Reproductive Physiology in Clinical Practice. Edited by Daniel H. Riddick. 277 pages, illustrated. New York, 1987, Thieme Medical Publishers, Inc. $59.95.

Strategies in Gynecologic Surgery. Edited by H. J. Buchsbaum and L. A. Walton. 231 pages, illustrated. New York, 1986, Springer-Verlag.

Surgical Lasers: A Clinical Guide. Edited by Terry A. Fuller. 237 pages, illustrated. New York, 1987, Macmillan Publishing Company. No price listed.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Premenstrual States: The Psychoanalytic/Psychobiologic Interface, October 17, 1987, Society for Ethical Culture Meeting House at Central Park West and 64th St., New York, New York, from 8:30 AM to 5:00 PM. Sponsored by the Columbia University Center for Psychoanalytic Training and Research in collaboration with the Association for Psychoanalytic Medicine. For further information contact: Columbia University Center for Psychoanalytic Training and Research, 722 West 168th St., New York, NY 10032. Tel.: (212) 927-5000.

Nineteenth Memphis Conference on the Mother, Fetus, and Newborn," September 17-18, 1987, The Peabody Hotel, Memphis, Tennessee. Sponsored by The University of Tennessee, Memphis, College of Medicine and Regional Medical Center at Memphis. For further information contact: Office of Continuing Medical Education, 800 Madison Avenue, Memphis, TN 38163. Tel.: (901) 528-5547.

First Congress of the International Society of Gynecological Endocrinology, Crans-Montana, Switzerland, March 6-12, 1988. For information contact the Scientific Secretariat/Organization: A. Volpe, F. Facchinetti, C. Nappi, or F. Petraglia at the Department of Obstetrics and Gynecology, University of Modena, Via del Pozzo, 71-41100 Modena, Italy. Tel.: (39) (59) 360224-360255-379515-379512.

Oral Examination: The American Board of Obstetrics and Gynecology, Inc. If you passed the written examination on or before June 30, 1986, you may be eligible to apply for the oral examination to be held in December 1988. The appropriate application form and current bulletin may be obtained by writing to the Office of the Executive Director, at the address shown below, in November-December 1987. The deadline for the receipt of applications in the Office of the Executive Director is February 28, 1988. To be ruled eligible, candidates must have unrestricted hospital privileges with independent unsupervised patient care. Candidates will be required to submit a list of all patients discharged from hospitals for a one-year span. Contact: James A. Merrill, M.D., Executive Director, The American Board of Obstetrics and Gynecology, Inc., 4507 University Way N.E., Suite 204, Seattle, WA 98105.

Problem Solving in Gynecologic Endocrinology and Infertility, December 11-12, 1987, The Knickerbocker Hotel, Chicago, Illinois. For information contact: The University of Chicago, Office of Continuing Medical Education, 5841 Maryland, Box 139, Chicago, IL 60637. Tel.: (312) 702-1056.

Association of Reproductive Health Professionals wishes to announce an educational seminar, "Women at Risk," November 20, 1987, Sheraton Boston Hotel, Boston, Massachusetts. For more information, please contact: ARHP, 900 Pennsylvania Ave., S.E., Washington, DC 20003.

International Workshop on Evaluating Medical Practice: The Case of Hysterectomy, January 13-15, 1988, Copenhagen, Denmark. For further information please contact: Myra Lewinter, Institute of Social Medicine, University of Copenhagen, Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark. Tel.: +45 1 35 79 00 (ext. 3011 or leave message on ext. 3010).

Group B Streptococcal Infection: "The Silent Epidemic," Wednesday, October 14, 1987, Woodbridge Hilton Hotel, Wood Ave. South, Iselin, New Jersey. For information contact the Department of Neonatology at Newark Beth Israel Medical Center. Tel.: (201) 926-7203.

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Krishna B. Singh, M.D.
Associate Professor and Chief
Section of Reproductive Endocrinology
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The Medical Center of Delaware
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Marshfield Clinic is seeking a board certified or eligible obstetrician/gynecologist to join its expanding regional center in Chippewa Falls. Chippewa Falls is a community of 15,000 people located in beautiful west central Wisconsin with a wide range of recreational, educational, and cultural opportunities easily accessible. The clinic is adjacent to a 110-bed JCAH accredited hospital. Marshfield Clinic is a 250-physician, multispecialty private group practice offering a very competitive salary and fringe benefit package. Send curriculum vitae and references to:

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Director, Regional Centers
Marshfield Clinic
1000 North Oak Avenue
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Or call collect at
(715) 387-5498

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John L. Duhring, M.D.
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Miami Beach, Florida 33140
Telephone No. (305) 674-2833

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GYNECOLOGIC ONCOLOGISTS

The Department of Reproductive Medicine, University of California, San Diego is seeking applicants for full time Assistant, Associate or full Professor faculty positions in the section of Gynecologic Oncology. One of the positions is that of Division Director. Activities will include patient care, research, and teaching at the medical student and postgraduate level. Qualified applicants should be Board Certified or Eligible (Active Candidate Status in general Obstetrics and Gynecology and Gynecologic Oncology. Appointment at the Assistant, Associate or full Professor level, as well as tenure track, is dependent upon previous experience. Salary related to rank and step of appointment is within the established salary schedule at the University of California, San Diego School of Medicine. Please send curriculum vitae and three names and addresses for recommendation before October 31, 1987 to:

Robert Resnik, M.D.
Professor and Chairman
Department of Reproductive Medicine
U.C.S.D. Medical Center
225 Dickinson Street
San Diego, California 92103

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Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
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Sacramento, California 95816

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Interested persons should send curriculum vitae to:

F. Gary Cunningham, M.D.
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University of Texas
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Dr. Thomas J. Gill III Chairman
Search Committee for Chairman of OB/GYN
University of Pittsburgh
School of Medicine
716 Scaife Hall
Pittsburgh, PA 15261

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Nashville, Tennessee 37232

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INDEX TO ADVERTISERS

AMI
Opportunity Available 64A
Ayerst Laboratories
Premarin 16A,17A,18A
Bay Shores Medical Group
Opportunity Available 69A
Bellwood General Hospital
Opportunity Available 61A
Berlex Laboratories, Inc.
Levlen 37A,38A
Tri-Levlen 20A,21A,22A
Boston University School of Medicine
Course 62A,64A
Ciba Pharmaceutical Company
Estraderm 53A,54A,55A,56A
Columbia University
Conference 49A
Del E. Webb Memorial Hospital
Opportunity Available 63A
FilmTec
Informed Consent Systems 32A
Flynn, Campbell, Collins' Executive Network
Opportunity Available 63A
Fort Collins Women's Clinic
Opportunity Available 63A
Friedrich Group
Opportunity Available 63A,66A
Grune & Stratton, Inc.
Book 32A
Hana Biologics, Inc.
Amniostat-FLM 39A
Hewlett Packard
Fetal Monitors 24A,25A
Hicks, C., D.
Opportunity Available 64A
Jefferson Medical College
Opportunity Available 71A
Kaiser Permanente
Opportunity Available 67A
Kent County Memorial Hospital
Opportunity Available 65A
Laconia Clinic
Opportunity Available 66A
Louisiana State University School of Medicine
Opportunity Available 66A
Marshfield Clinic
Opportunity Available 61A,65A,67A
Matthew Thornton Health Plan
Opportunity Available 65A
Mead Johnson Laboratories
Natalins Rx 12A
Medical Center of Delaware
Opportunity Available 66A
Medical College of Ohio
Opportunity Available 68A
Merck Sharp & Dohme
Mefoxin 30A,31A,32A
Michael Reese Health Plan
Opportunity Available 67A
Mount Sinai Medical Center
Opportunity Available 68A
Multigon Industries, Inc.
Doppler Spectrum Analyzer 14A
National Institutes of Health
Fellowship 62A
Orlando Regional Medical Center
Opportunity Available 68A
Ortho Pharmaceutical Corporation
All-Flex 10A
Ortho-Novum 7/7/7 ..... Third Cover,Fourth Cover
Osler Institute
Ob-Gyn Review 76A
Oxford University Press
Journal 49A
Park City Hospital, The
Opportunity Available 68A
Perinatology Press
Book 61A
Radiology Postgraduate Education
Book 40A
Searle & Co.
Demulen 1/35 ..... 33A,34A,35A,36A,45A,46A,47A, 48A,57A,58A,59A,60A
Smith Kline & French Laboratories
Cefizox 50A,51A,52A
Southboro Medical Group, Inc.
Opportunity Available 69A
St. Luke's Hospital of Kansas City
Opportunity Available 69A
State University of New York at Stony Brook
Opportunity Available 69A
Syntex Laboratories, Inc.
Femstat 41A,42A,43A,44A
Tri-Norinyl ..... Second Cover,1A,2A
Trabin, Manko and Litt
Opportunity Available 70A
Truman Medical Center
Opportunity Available 70A
U.C.S.D. Medical Center
Opportunity Available 70A
University of California, Davis, Medical Center
Opportunity Available 71A
University of Lausanne
Opportunity Available 71A
University of Pittsburgh
Opportunity Available 72A
University of Texas
Opportunity Available 71A
Upjohn Company, The
Provera 3A,4A
Vanderbilt University Medical Center
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OBSTETRICS AND GYNECOLOGY REVIEW

honoring Robert Scully, M.D.

*Any Six Days — December 1-9, 1987 — Chicago

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OBJECTIVES

- Increase basic knowledge in obstetrics and gynecology
- Improve clinical skills in diagnosis and treatment
- Assist residents and fellows in organizing study
- Prepare board candidates to pass board examinations
- Provide practicing surgeons with a review and update

METHODS

- HOME STUDY MATERIALS with questions and ansv based on previous examinations
- SEMINAR with projection slides and syllabus
- LABORATORY with microscopic slides
- PRACTICE EXAMS with oral and written parts

* Dec. 1-6 (live); Dec. 7-9 (Video replay of Dec. 1-3). Any consecutive six days will complete program.

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- Vulva
- Vagina
- Cervix
- Endometrium
- Myometrium
- Ovary
- Placenta

BASIC SCIENCES
- Anatomy
- Embryology
- Genetics
- Physiology
- Pharmacology
- Biochemistry
- Legal Problems

CLINICAL SCIENCES
- Anesthesiology
- Clinical Laboratory
- Endocrinology
- Infectious Disease
- Psychology
- Radiology
- Radiotherapy

OBSTETRICS
- Pregnancy
- Labor
- Delivery
- Puerperium
- Abortion
- Fetal Development
- Neonatology

GYNECOLOGY
- Pelvic Relaxation
- Menstruation
- Contraception
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- Endometriosis
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**Comments by participants of our previous cou*

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MPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the roduct and therefore should not be used as the basis for prescribing the product. This summary was prepared by eleting from the complete prescribing information certain text, tables, and references. The physician should be oroughly familiar with the complete prescribing information before prescribing the product.

NDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

OSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive ssociation between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is rudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive roduct prescribed for any given patient should be that product which contains the least amount of estrogen that is ompatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral ontraceptives be started on preparations containing .05 mg or less of estrogen.

ONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. hrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic isorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or uspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may ause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who re pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard o the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral ontraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and hrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great ritain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism nd stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times nore likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary mbolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **EREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in onusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study onducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than hat of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study onducted in the United States found that past use of oral contraceptives was associated with increased risk of ubarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk nay be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial nfarction associated with the use of oral contraceptives has been reported confirming a previously suspected ssociation. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of nderlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, iabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether he patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk actor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive sers was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 00,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as ikely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold ncreased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. n determining the importance of these relative risks, however, the baseline rates for various age groups must be given erious consideration. The importance of other predisposing conditions mentioned above in determining relative and bsolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a esser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists ollowing discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British nvestigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher isk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an ndependent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. Cases of hepatocellular carcinoma, although rare, have been reported in women taking oral contraceptives. Retrospective studies conducted in the United States and Britain report an increased risk of hepatocellular carcinoma in oral contraceptive users, particularly with increased duration of use. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **DRUG/LABORATORY TEST INTERACTIONS:** The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

ORTHO

Beyond low dose...

EXCELLENT RESULTS

Low incidence of nuisance side effects†
Low breakthrough bleeding
Low impact on selected metabolic parameters

21- and 28-day regimens

ORTHO-NOVUM 7/7/7 Tablets

TRADEMARK

norethindrone/ethinyl estradiol

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see brief summary of complete Prescribing Information on the preceding page.

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ORTHO

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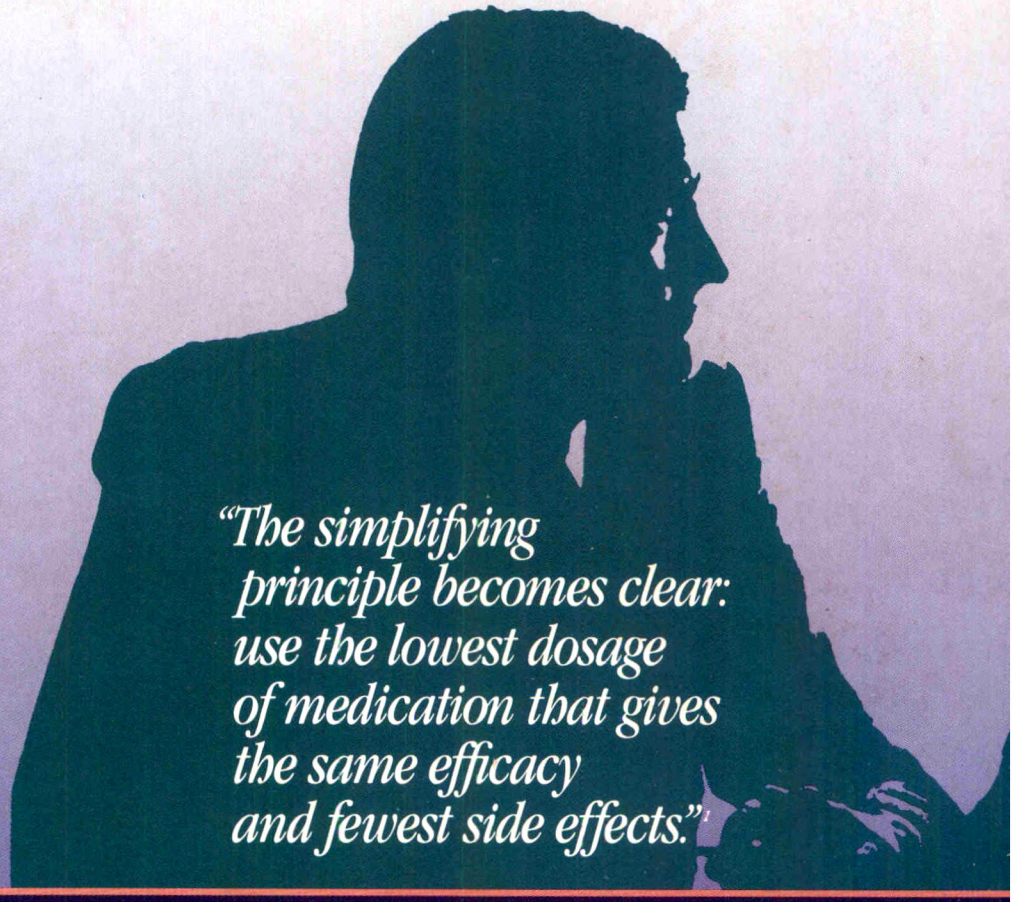
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Today's authoritie



"The simplifying principle becomes clear: use the lowest dosage of medication that gives the same efficacy and fewest side effects."[1]

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- 29% less progestin per cycle than a 1/35 formulation[2*]

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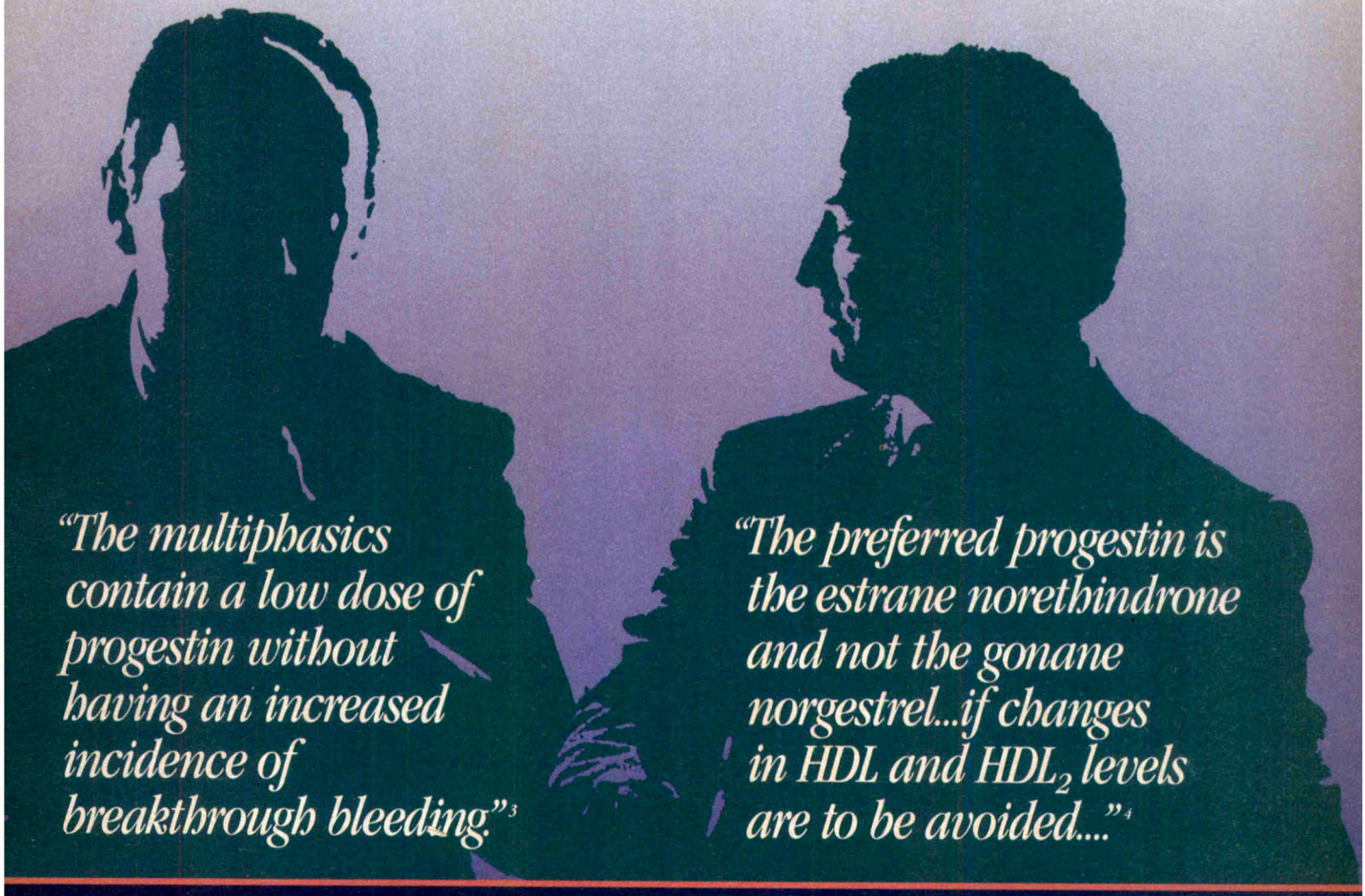
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2. Brett L: *Clinical Evaluation of Tri-Norinyl, a Triphasic Oral Contraceptive.* Palo Alto, CA, Syntex Laboratories, Inc., 198
3. Roundtable discussion. *Dialogues in Contraception* 1986;6:4.
4. Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol* 1982;142:766-772.

*1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Comparative data available from Syntex Laboratories, Inc.

No endorsement of Tri-Norinyl or any other Syntex oral contraceptive has been given by the authorities cited.

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...on oral contraception:



"The multiphasics contain a low dose of progestin without having an increased incidence of breakthrough bleeding."[3]

"The preferred progestin is the estrane norethindrone and not the gonane norgestrel...if changes in HDL and HDL_2 levels are to be avoided...."[4]

Low BTB with Tri-Norinyl

- Extra midcycle protection that's unique among multiphasics
- Low BTB rates—equivalent to a fixed low-dose OC[2]

Low Impact with Tri-Norinyl

- Minimal lipid and cholesterol impact[4]
- Low incidence of nuisance side effects[2]

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

THE LOWEST-DOSE MULTIPHASIC OC WITH NORETHINDRONE

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of prescribing information on next page.

TRI - NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34 – 5/100,000; ages 35-44 – 33/100,000; ages 45-49 – 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 – 1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over – 1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS – RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. (See also Warnings, 4. *Liver Tumors.*) 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. Cases of hepatocellular carcinoma have been reported in long-term users of OCs.[105-107] An epidemiological study suggested a 4-fold increase in relative risk for hepatocellular carcinoma in OC users for 8 years or more, which increased to 7 in women with markers of hepatitis B. A second study suggested a risk of about 20 for use in excess of 8 years. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones – estrogenic and progestational agents – during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero.* These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests that the incidence of *Chlamydia trachomatis* infection, the leading cause of nongonococcal PI.D., is increased by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Budd-Chiari syndrome, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure, premature supraventricular contractions. **Information for the Patient** (See Patient Package Insert).

Rev. April 1987

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

Caution: Federal law prohibits dispensing without prescription.

MED B-13-S

Upjohn 100

A Century of Caring
1886·1986

March 1986

J-6144

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The Upjohn Company, Kalamazoo, MI 49001

A Standard



Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.

MOSBY

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Founded in 1920

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Contents

October
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1987

CLINICAL SECTION

Clinical Opinion

Risk scoring for preterm labor: Where do we go from here? 789
Denise M. Main, M.D., and Steven G. Gabbe, M.D.
Philadelphia, Pennsylvania

Prophylactic antibiotics for cesarean delivery: A simple cost-effective strategy for prevention of postoperative morbidity 794
Patrick Duff, M.D.
Tacoma, Washington

Clinical Articles

Presidential address of Association of Professors of Gynecology and Obstetrics 799
Allan B. Weingold, M.D.
Washington, D. C.

(Contents continued on page 7A)

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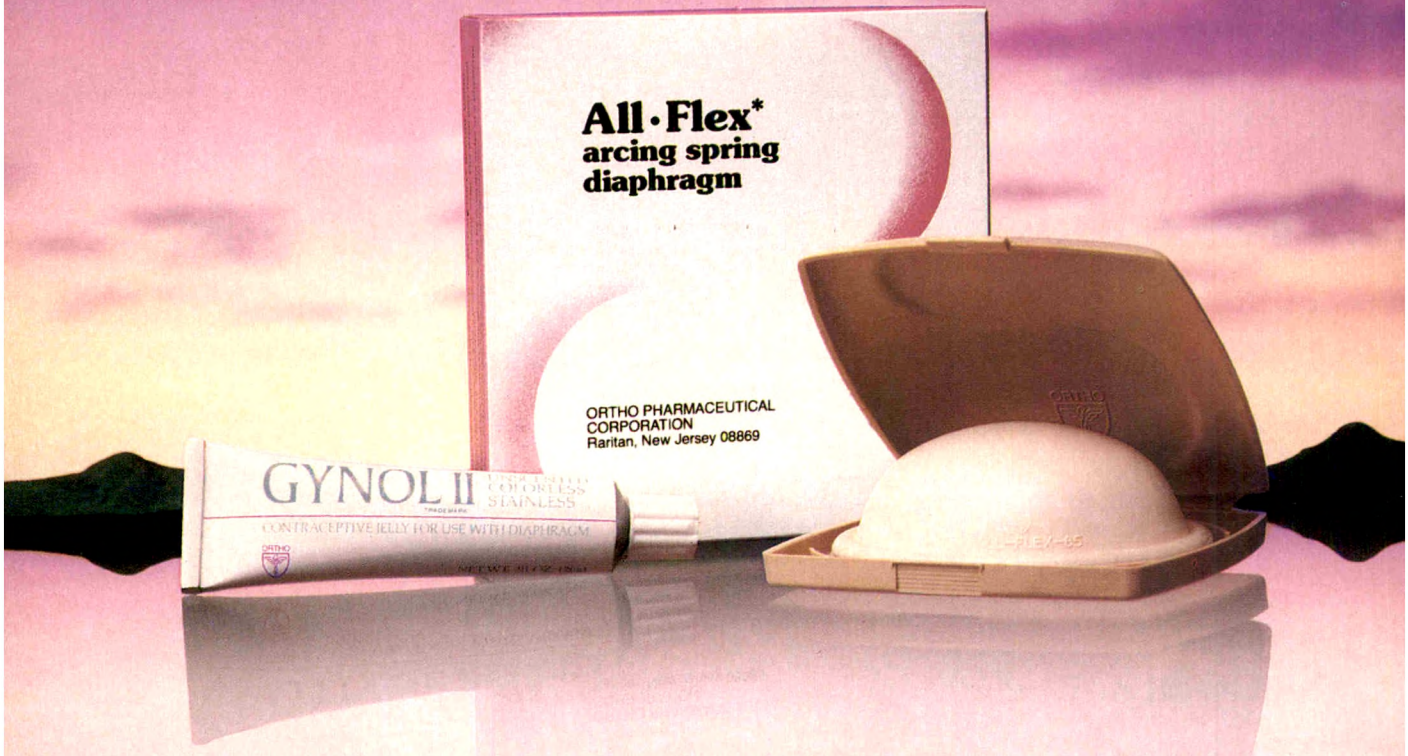
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Contents continued from page 5A

A sonographic screening method for Down syndrome 803
C. Lockwood, M.D., B. Benacerraf, M.D., A. Krinsky, M.D., K. Blakemore, M.D., K. Belanger, Ph.D., M. Mahoney, M.D., and J. Hobbins, M.D.
New Haven, Connecticut, and Boston, Massachusetts

Colposcopic correlates of cervical papillomavirus infection 809
Michele M. Follen, M.D., Richard U. Levine, M.D., Ellen Carillo, R.N., Ralph M. Richart, M.D., Gerard Nuovo, M.D., and Christopher P. Crum, M.D.
New York, New York

Infection and labor: The detection of endotoxin in amniotic fluid 815
Roberto Romero, M.D., Nicholas Kadar, M.D., John C. Hobbins, M.D., and Gordon W. Duff, M.D., Ph.D.
New Haven, Connecticut

Maternal serum α-fetoprotein screening. III. Pitfalls in evaluating black gravid women 820
James N. Macri, Ph.D., Ramana V. Kasturi, B.E., M.S., Mannshya G. Hu, Ph.D., David A. Krantz, B.S., Timothy J. Douros, Paul Sajda, and Edward J. Cook, Ph.D.
Carle Place, New York

Amniotic fluid α-fetoprotein levels and pregnancy outcome 822
Cynthia G. Brumfield, M.D., Gretchen A. Cloud, M.S., Sara C. Finley, M.D., Paula Cosper, Ph.D., Richard O. Davis, M.D., and John F. Huddleston, M.D.
Birmingham, Alabama

Cord serum bromide concentration: Variation and lack of association with pregnancy outcome 826
Marvin E. Miller, M.D., Janice M. Cosgriff, B.S., and Klaus J. Roghmann, Ph.D.
Rochester, New York

Fetal-induced trauma as a cause of antepartum hemorrhage 830
John A. Eden, M.R.C.O.G.
London, England

A pregnant woman with a high level of naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii* 832
Eiji Konishi, Ph.D.
Kobe, Japan

The umbilical cord twist: Origin, direction, and relevance 833
Ronald V. Lacro, M.D., Kenneth L. Jones, M.D., and Kurt Benirschke, M.D.
San Diego, California

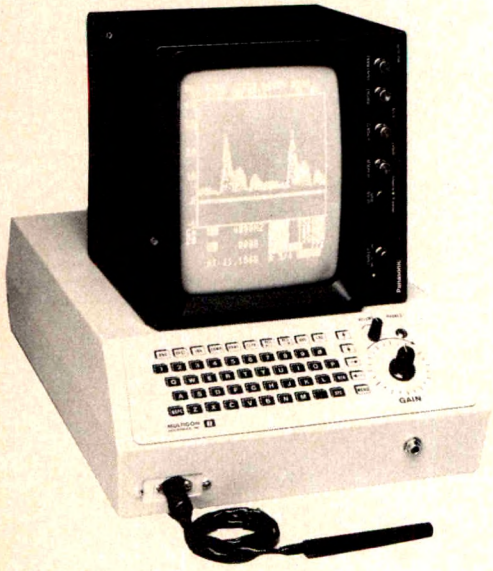
(Contents continued on page 9A)

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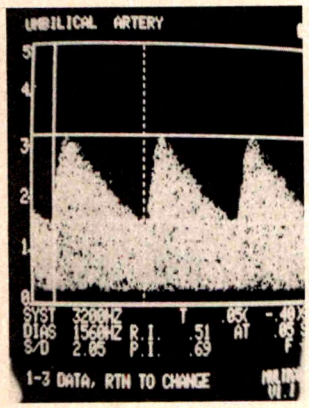


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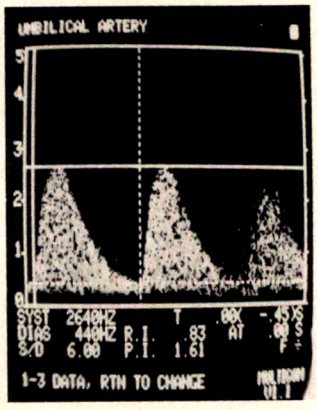
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Contents continued from page 7A

Indicators of perinatal asphyxia 839
David I. Hollander, M.D., Linda Wright, M.D., David A. Nagey, M.D., Ph.D., John N. Wright, Ph.D., Marcos J. Pupkin, M.D., and Thomas Koch, Ph.D.
Baltimore, Maryland

Umbilical cord blood pH and Apgar scores as an index of neonatal health 843
Bruce E. Josten, Captain, USAF, MC, Timothy R. B. Johnson, Major, USAF, MC, and John P. Nelson, Major, USAF, BSC
Andrews Air Force Base, Maryland

Cervical endometriosis: Facilitated diagnosis by fine needle aspiration cytologic testing 849
Maria M. Veiga-Ferreira, M.D., Gladwyn Leiman, M.B., B.Ch., Fiona Dunbar, M.B., B.Ch., and Karin A. Margolius, F.C.Path.(S.A.)
Johannesburg, South Africa

A novel Y/13 familial translocation 857
Michael I. Morris, Ph.D., Frederick W. Hanson, M.D., and Frances R. Tennant, Ph.D.
Sacramento, California

In vitro training model for diagnostic and therapeutic fetal intravascular needle puncture 858
Ilan E. Timor-Tritsch, M.D., and Ming-Neng Yeh, M.D.
New York, New York

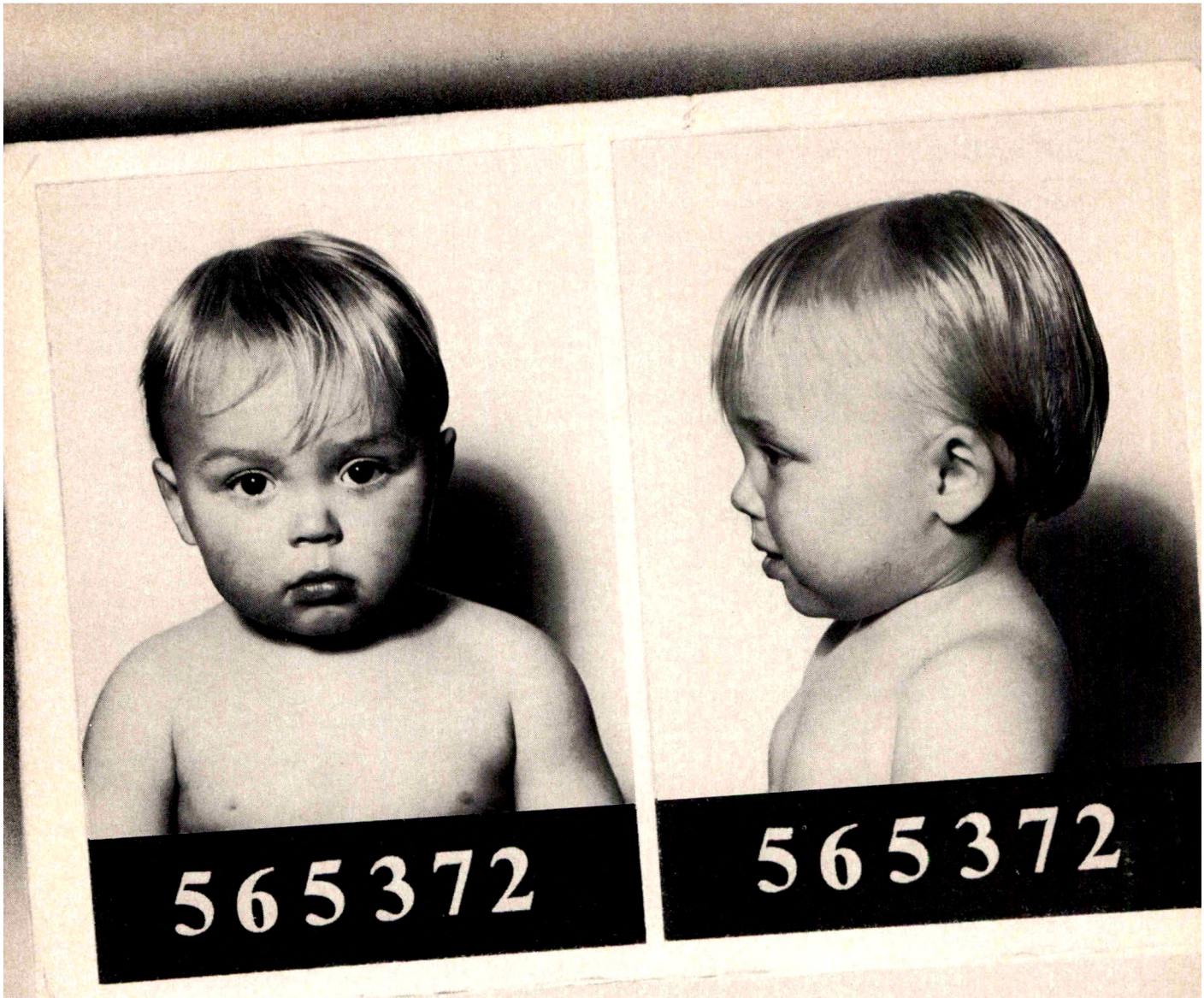
Antepartum fetal surveillance in the patient with decreased fetal movement 860
Myoung Ock Ahn, M.D., Jeffrey P. Phelan, M.D., Carl Vernon Smith, M.D., Nancy Jacobs, R.N., and Susan E. Rutherford, M.D.
Los Angeles, California

Validity of intrapartum diagnosis of fetal thrombocytopenia 864
Godelieve C. M. L. Christiaens, M.D., Ph.D., and Frans M. Helmerhorst, M.D., Ph.D.
Utrecht and Leiden, The Netherlands

Effects of maternal glucose infusion on fetal acid-base status in human pregnancy 866
Elliot H. Philipson, M.D., Satish C. Kalhan, M.B., Margo M. Riha, R.N., and Renato Pimentel, M.D.
Cleveland, Ohio

The use of fetal neuromuscular blockade during intrauterine procedures 874
Kenneth J. Moise, Jr., M.D., Robert J. Carpenter, Jr., M.D., Russell L. Deter, M.D., Brian Kirshon, M.D., and Sal F. Diaz, M.D.
Houston, Texas

(Contents continued on page 11A)



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Contents continued from page 9A

Fetal assessment based on fetal biophysical profile scoring: Experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths 880
F. A. Manning, M.D., I. Morrison, M.B., C. R. Harman, M.D., I. R. Lange, M.B., and S. Menticoglou, M.D.
Winnipeg, Manitoba, Canada

Acute oligohydramnios in postdate pregnancy 884
Daniel Clement, M.D., Barry S. Schifrin, M.D., and Richard B. Kates, M.D.
Tarzana, California, and Spokane, Washington

Abdominal pregnancy: Magnetic resonance identification with ultrasonographic follow-up of placental involution 887
Rahima Spanta, M.D., Larry E. Roffman, M.D., Timothy J. Grissom, M.D., James R. Newland, M.D., and Bruce M. McManus, M.D., Ph.D.
Omaha, Nebraska

Preterm birth at 23 to 26 weeks' gestation: Is active obstetric management justified? 890
Chukwuma G. Nwaesei, M.D., David C. Young, M.D. Joseph M. Byrne, Ph.D., Michael J. Vincer, M.D., Doris Sampson, R.N., Jacquelyn R. Evans, M.D., Alexander C. Allen, M.D., and Dora A. Stinson, M.D.
Halifax, Nova Scotia, Canada

Inhibitory effect of quinidine on plasma pseudocholinesterase activity in pregnant women 897
J. Reddy Kambam, M.D., John J. Franks, M.D., and Bradley E. Smith, M.D.
Nashville, Tennessee

Umbilical artery flow velocity waveforms and the placental vascular bed 900
Lesley M. McCowan, M.B., Brendan M. Mullen, M.D., and Knox Ritchie, M.D.
Toronto, Ontario, Canada

Adenosquamous carcinoma of the fallopian tube 903
David H. Moore, M.D., John T. Woosley, M.D., Ph.D., Robert L. Reddick, M.D., M.S., Leslie A. Walton, M.D., and Gene P. Siegal, M.D., Ph.D.
Chapel Hill, North Carolina

Increased tubular enzyme excretion in preeclampsia 906
Marshall P. Goren, M.D., Baha M. Sibai, M.D., and Adel El-Nazar, M.D.
Memphis, Tennessee

Short course of antibiotics for post–cesarean section endometritis 908
Michael Cabbad, M.D., Odie Sijin, B.S., and Howard Minkoff, M.D.
Brooklyn, New York

(Contents continued on page 12A)

Contents continued from page 11A

First-trimester serum levels of the β-subunit of human chorionic gonadotropin in a tubal molar pregnancy 910
Jeffrey S. Chase, M.D., Jerome H. Check, M.D., Khosrow Nowroozi, M.D., and Chung H. Wu, M.D.
Philadelphia, Pennsylvania

Pregnancy in Henoch-Schönlein purpura 911
Geetha Joseph, M.D., James S. Holtman, M.D., Rodney E. Kosfeld, M.D., William A. Blodgett, M.D., and Yong K. Liu, M.D.
Louisville, Kentucky

Theca-lutein ovarian cysts associated with homozygous α-thalassemia 912
Philip D. Miller, M.D., Brenda C. Smith, M.D., and David N. Marinoff, M.D.
Santa Clara, California

Racial differences in survival of women with endometrial cancer 914
Raymond P. Bain, Ph.D., Raymond S. Greenberg, M.D., Ph.D., and Kevin C. Chung, B.A.
Atlanta, Georgia

Endometrial adenocarcinoma estrogen receptor content: Association of clinicopathologic features with immunohistochemical analysis compared with standard biochemical methods 924
David G. Mutch, M.D., John T. Soper, M.D., Debra A. Budwit-Novotny, M.D., Edwin B. Cox, M.D., William T. Creasman, M.D., K. S. McCarty, Sr., Ph.D., and K. S. McCarty, Jr., M.D., Ph.D.
Durham, North Carolina

Colposcopic evaluation of abnormal Papanicolaou smears in pregnancy 932
J. L. Benedet, M.D., P. A. Selke, M.D., and K. G. Nickerson, M.D.
Vancouver, British Columbia, Canada

Acute effects of exercise on plasma catecholamines in sedentary and athletic women with normal and abnormal menses 938
NeeOo W. Chin, M.D., Frank E. Chang, M.D., William G. Dodds, M.D., Moon H. Kim, M.D., and William B. Malarkey, M.D.
Columbus, Ohio

Treatment of menorrhagia with meclofenamate sodium 944
Joyce M. Vargyas, M.D., Joseph D. Campeau, M.S., and Daniel R. Mishell, Jr., M.D.
Los Angeles, California

Configuration of the prepubertal hymen 950
Susan Ferrell Pokorny, M.D.
Houston, Texas

Contents

Suppression of lymphocyte reactivity in vitro by supernatants of explants of human endometrium 956
Huei-Sheng Wang, M.D., Hideharu Kanzaki, M.D., Masumi Yoshida, M.D., Shigeyasu Sato, M.D., Makoto Tokushige, M.D., and Takahide Mori, M.D.
Kyoto, Japan

Magnesium and zinc status during the menstrual cycle 964
Patricia A. Deuster, Ph.D., Eran Dolev, M.D., Lani L. Bernier, B.S., and Ulrike H. Trostmann, M.S.
Bethesda, Maryland

Partial hydatidiform moles: Deoxyribonucleic acid content and course 969
John R. Davis, M.D., Daniel P. Kerrigan, M.D., Dennis L. Way, B.S., and Sheldon A. Weiner, M.D.
Tucson, Arizona

Correlation between chlamydia infection and clinical evaluation, vaginal wet smear, and cervical swab test in female adolescents 974
Henning Thejls, M.D., Vivi Anne Rahm, Gerd Rosen, and Håkan Gnarpe, M.D., Ph.D.
Gävle, Sweden

BASIC SCIENCE SECTION

Evaluation of 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: A new tocolytic agent 977
D. W. Hahn, Ph.D., K. T. Demarest, Ph.D., E. Ericson, A.S., R. E. Homm, B.S., R. J. Capetola, Ph.D., and J. L. McGuire, Ph.D.
Raritan, New Jersey

Stereotypic behavioral response of rat fetuses to acute hypoxia is altered by maternal alcohol consumption 982
William P. Smotherman, Ph.D., and Scott R. Robinson, M.S.
Corvallis, Oregon

Effects of norepinephrine on lung fluid flow rate in the chronically catheterized fetal lamb 986
Motokazu Higuchi, M.D., Yuji Murata, M.D., Yoshiaki Miyake, M.D., John Hesser, M.D., James Tyner, Kirk A. Keegan, M.D., and Manuel Porto, M.D.
Irvine, California

(Contents continued on page 14A)

Contents continued from page 13A

Characteristics of the electromyogram recorded from the mesometrium of the pregnant ewe from 106 days' gestation to delivery: Similarities with and differences from the electromyogram obtained from the myometrium 991
J. P. Figueroa, M.D., A. Massmann, M.D., G. Pimentel, D.V.M., and P. W. Nathanielsz, M.D., Ph.D.
Ithaca, New York

β-Adrenergic receptor agonist infusion increases plasma prostaglandin F levels in pregnant sheep 998
Robert F. Casper, M.D., and Stephen J. Lye, Ph.D.
London, Ontario, Canada

Nifedipine: Effects on fetal and maternal hemodynamics in pregnant sheep 1003
Bilal Harake, M.D., Raymond D. Gilbert, Ph.D., Stephen Ashwal, M.D., and Gordon G. Power, M.D.
Loma Linda, California

CORRESPONDENCE

Absence of clomiphene and/or active metabolites in blood of clomiphene citrate–treated patients during organogenesis: Determination by radioreceptor assay Avraham Geier, Ph.D., Josef Blankstein, M.D., Ehud Kokia, M.D., Clara Pariente, M.Sc., and Bruno Lunenfeld, M.D., *Tel-Hashomer, Israel* 1009

Spontaneous fetal seizures in utero P. G. Maouris, M.D. *Manchester, England* 1009

Human ovarian oxytocin and steroid hormones Frederick J. Auletta, Ph.D., and A. P. F. Flint, Ph.D. *Cambridge, England* 1010

Reply Firyal S. Khan-Dawood, Ph.D., and M. Yusoff Dawood, M.D. *Chicago, Illinois* 1011

Teen pregnancies Gregory R. Roush *Canton, Ohio* 1011

Reply Dorothy Hollingsworth, M.D., and Marianne E. Felice, M.D. *San Diego, California* 1011

Have Liley charts outlived their usefulness? John L. Duhring, M.D. *Toledo, Ohio* 1011

Reply Kypros Nicolaides, M.D. *London, England* 1012

Hypertensive diseases of pregnancy: Nulliparity, primiparity, multiparity, and gravidity Kenneth J. Reamy, M.D., M.P.H. *Morgantown, West Virginia* 1012

Reply Norbert Gleicher, M.D. *Chicago, Illinois* 1012

Pemphigus in pregnancy: A reevaluation of fetal risk A. Metzker, M.D., and P. Merlob, M.D. *Petah-Tiqva, Israel* 1012

The cause of uterine softening in pregnancy Ákos A. Jakobovits, M.D., László Zubek, M.D., Ph.D., Géza Pusztai, M.D., Tibor Albu, M.D., and Csaba Harmos, M.D. *Cegléd, Hungary* 1013

Intrapartum electronic fetal monitoring efficacy Sylvain Fribourg, M.D. *Panorama City, California* 1013

Reply Stephen B. Thacker, M.D. *Atlanta, Georgia* 1014

Contents

Previous cesarean delivery and trial of labor Sylvain Fribourg, M.D. *Panorama City, California* 1014

Reply Brent E. Finley, M.D. *Kansas City, Kansas* 1015

HELLP syndrome J. R. Gerace, M.D. *Corona, California* 1015

Reply Baha M. Sibai, M.D. *Memphis, Tennessee* 1016

BOOKS

Books received 1017

ITEMS

Items 1018

Information for authors on pages 23A, 24A, and 25A

Index to advertisers on page 58A

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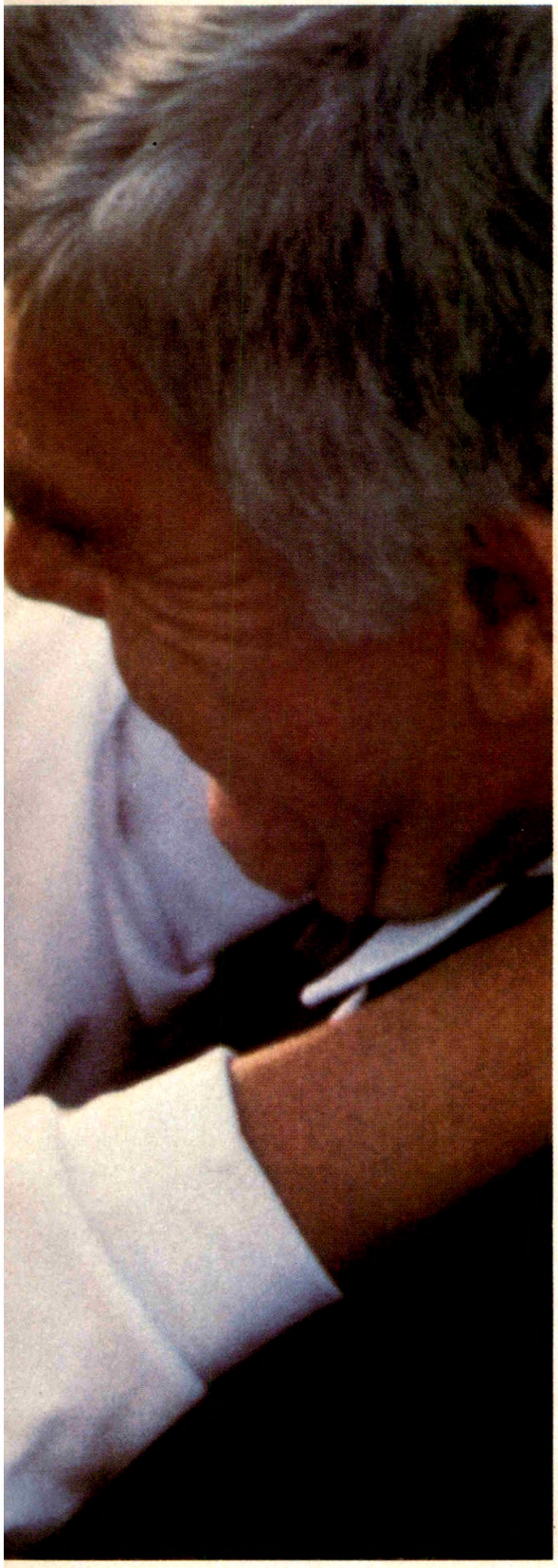
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Three independent, case-controlled studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case-controlled studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semi-annual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equi-estrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing, in later life, a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30% to 90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case-controlled study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: PREMARIN (conjugated estrogens tablets, USP): Moderate-to-severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms and they should not be used to treat such conditions.) Osteoporosis (abnormally low bone mass). Atrophic vaginitis. Kraurosis vulvae. Female castration.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Concomitant Progestin Use: The lowest effective dose appropriate for the specific indication should be utilized. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of the endometrium suggest that 10 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks which may be associated with the inclusion of progestin in estrogen replacement regimens. (See PRECAUTIONS.) The choice of progestin and dosage may be important; product labeling should be reviewed to minimize possible adverse effects.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (see Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Estrogens have been reported to increase the risk of endometrial carcinoma (see Boxed Warning). However, a recent large, case-controlled study indicated no increase in risk of breast cancer in postmenopausal women. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not yet complete. If concomitant progestin therapy is used, potential risks may include adverse effects on carbohydrate and lipid metabolism.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. However, in a recent, large case-controlled study of postmenopausal women there was no increase in risk of breast cancer with use of conjugated estrogens.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.

2. *Given cyclically:* Osteoporosis. Female castration. Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off). Female castration—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (eg, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

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1. Lindsay R, Hart DM, Clark DM: The minimum effective dose of estrogen for prevention of postmenopausal bone loss. *Obstet Gynecol* 1984;63:759-763. **2.** Studd JWW, Thom MH, Paterson MEL, et al: The prevention and treatment of endometrial pathology in postmenopausal women receiving exogenous estrogens, in Pasetto N, Paoletti R, Ambrus JL (eds): *The Menopause and Postmenopause*. Lancaster, England, MTP Press Ltd, 1980, chap 13.

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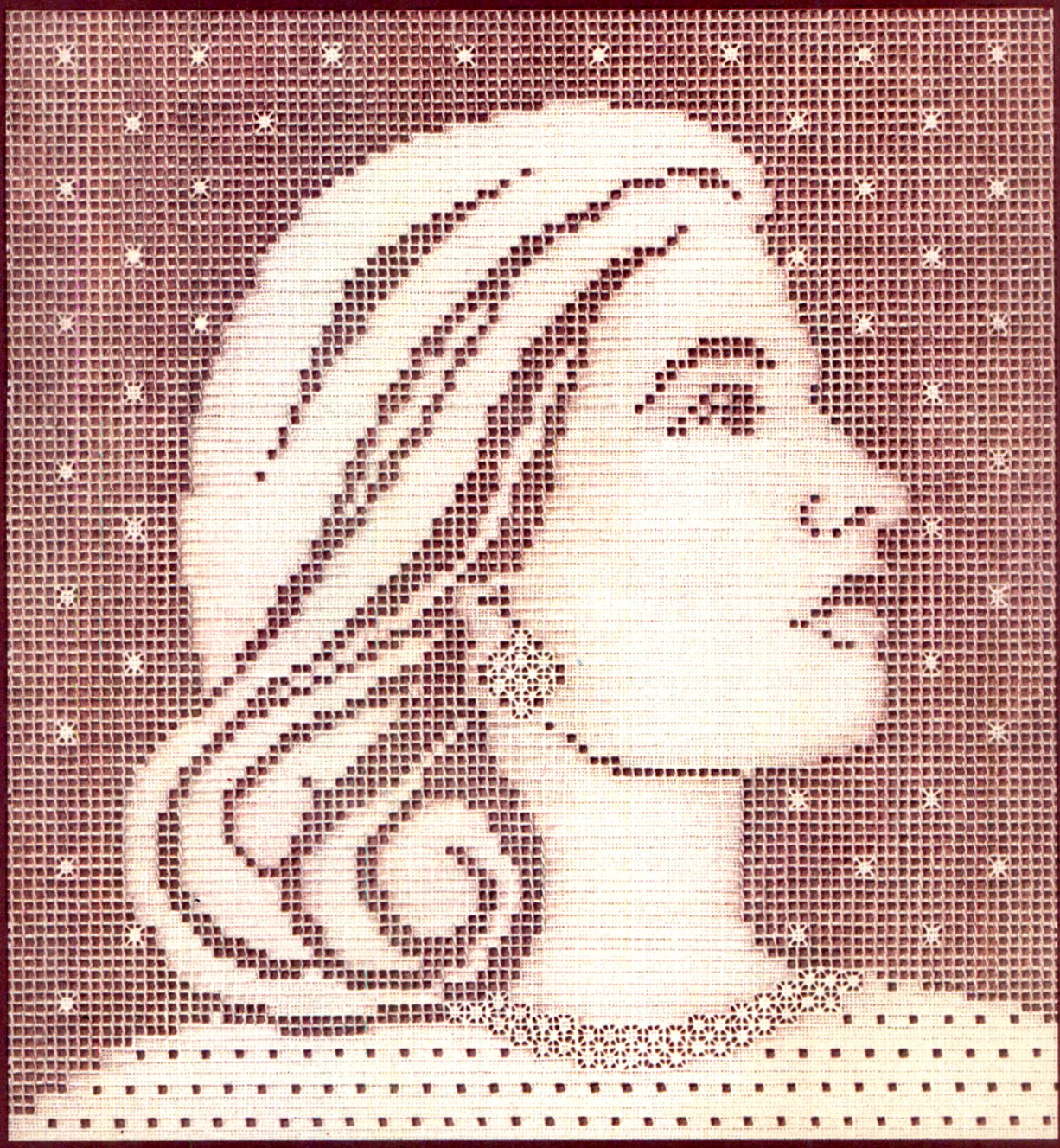
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An enduring pattern of excellence



LEVLEN™

(Levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg tablets)

The lowest total hormonal dose in a monophasic OC formula

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. The physician should be aware of the complete prescribing information for this product. Please also see brief summary of prescribing information on the next page.

BERLEX™ Dedicated to innovation in OB/GYN medicine

LEVLEN™ 28
LEVLEN™ 21

(Levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg tablets)

BRIEF SUMMARY

Each Levlen tablet contains 0.15 mg of levonorgestrel (d (-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.03 mg of ethinyl estradiol (19-nor-17 α-pregna-1,3,5 (10)-trien-20-yne-3,17-diol) (7 pink inert tablets are included in Levlen 28).

Indications and Usage—Levlen is indicated for the prevention of pregnancy in women who elect to use OCs as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only OCs have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. (The rates given for Pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutiv periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribe schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the la menstrual period if the progestogen-only OCs are used), and further use of OCs should be withheld until pregnancy has been ruled ou If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of th pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinician recommend 3 months, although no precise information is available on which to base this recommendation. The administration progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnanc

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, a increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase triglycerides and total phospholipids has been observed in patients receiving OCs. The clinical significance of these findings remain to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertensio may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in use and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposur and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated wit the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely t develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to norma after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule ou pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing t an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessar since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only O failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving OCs, since the drugs are mor effective in preventing intrauterine than ectopic pregnancies.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity an quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should b deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatmen and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 yea without another physical examination being performed.

2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patient becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in a attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should b prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundic during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patien receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients wit impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in norma tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8 Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See patient package labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs:

a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3 increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use o rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline

Carcinogenesis, Mutagenesis, Impairment of Fertility—See "Warnings" section #3, 4 and 5 for information on carcinogenesis mutagenesis and impairment of fertility.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings." Because of the potential for adverse reactions in nursing infants from OC tablets, a decisior should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (se "Warnings"): thrombophlebitis; pulmonary embolism; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertensior gallbladder disease; benign hepatomas; congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies ar needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/o vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Othe reactions, as a general rule, are seen much less frequently or only occasionally; gastrointestinal symptoms (such as abdominal cramp and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness enlargement and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possibl diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening) intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; ner vousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis porphyria; hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Levlen must be taken exactly as directed and at interval not exceeding 24 hours.

Any time the patient misses 1 or 2 light-orange tablets, she should also use another method of contraception until she has taken light-orange tablet daily for 7 consecutive days.

For full details on dosage and administration, see prescribing information in package insert.



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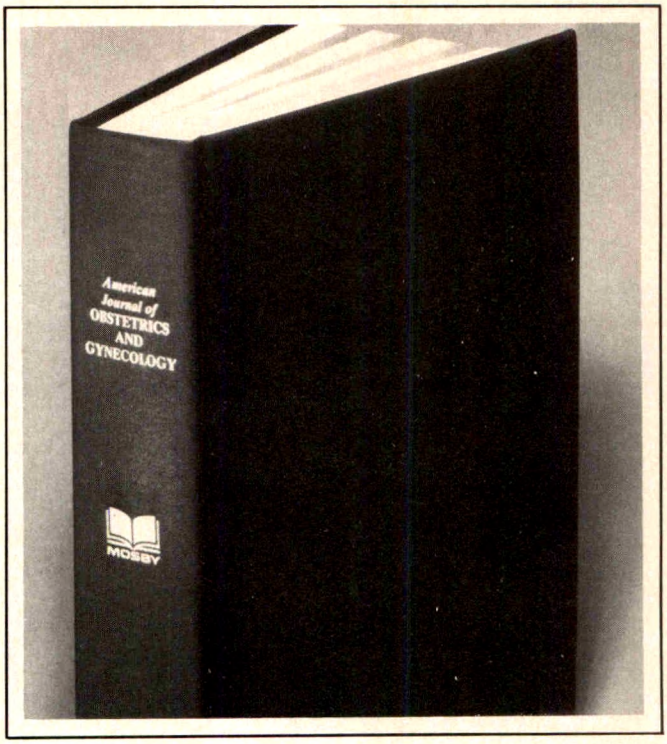
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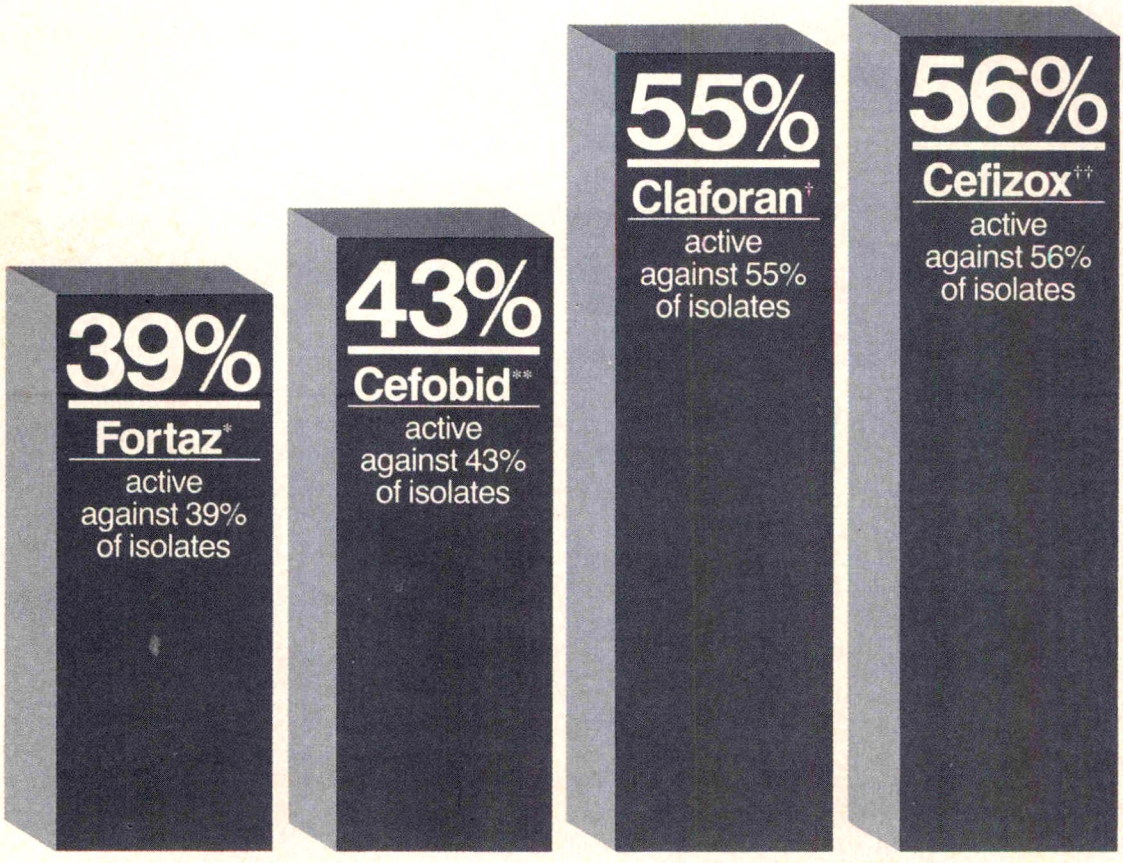
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NOTE: *In vitro* activity does not necessarily imply *in vivo* effectiveness.

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GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST II reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk: Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials, infusion bottles, and ADD-Vantage™ # vials containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*

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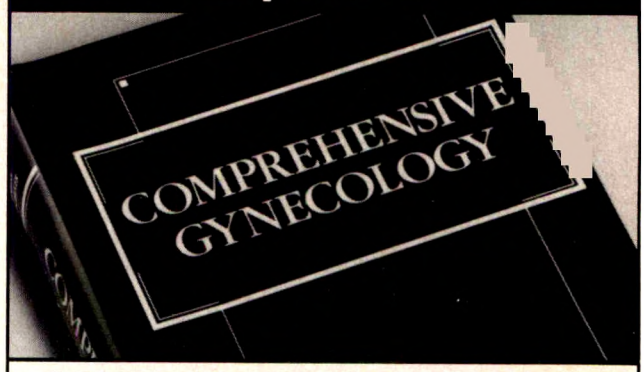
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CLINICAL SECTION

Clinical Opinion

Risk scoring for preterm labor: Where do we go from here?

Denise M. Main, M.D., and Steven G. Gabbe, M.D.

Philadelphia, Pennsylvania

Risk scoring systems based on traditional demographic, social, and medical factors have limited use, especially in certain populations. Newer approaches, including frequent cervical examinations, periodic outpatient monitoring for contractions, extensive daily monitoring at home for contractions in very high-risk persons, and identification and reduction of modifiable behavioral risks, offer promise. (AM J OBSTET GYNECOL 1987;157:789-93.)

Key words: Risk assessment for preterm birth

Enthusiasm for risk scoring systems based primarily on traditional epidemiologic and medical factors to predict spontaneous preterm birth peaked in the 1970s and early 1980s. It was widely assumed that scoring systems could be developed that would effectively identify women at increased risk for spontaneous preterm delivery. Specific interventions designed to diagnose preterm labor early could then be selectively applied to women identified as at high risk.

Established risk scoring indexes

Five risk scoring systems have been developed to predict spontaneous preterm birth[1-7] (Tables I and II). Although varying in detail, these systems consist of selected social and demographic factors, past obstetric and medical history, and in some cases current pregnancy complications. The most widely used system is that developed by Papiernik and modified by Creasy. It is easy to apply, specific in its goal to identify women at high risk for spontaneous preterm birth, and has performed better than other scores in prospective studies. Thirteen percent of a population screened in New Zealand were determined to be at high risk. These women had a 30% chance of delivering preterm infants (positive predictive value) and accounted for 64% of all the spontaneous preterm births in the population. The sensitivity of the screen was much higher for multigravidas than for primigravidas (77% versus 31%). This screen has performed less well in United States samples.[5,6]

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Limitations of established risk scoring systems

One of the major limitations of these risk assessment tools results from our relative ignorance about the pathophysiology of preterm labor and premature rupture of the membranes. Multiple factors contribute to these common outcomes. It is unlikely that traditional risk factors can achieve useful specificity and sensitivity in predicting some causes such as abruptio placentae or genital tract infections. In the best situation, the risk scoring indexes identify two thirds of the women who experience preterm birth. Some factors that appear to be very predictive, such as diethylstilbestrol exposure or uterine malformation, occur very infrequently and hence have low attributable risk within a population. In contrast, other factors such as smoking, which as an individual risk factor is a weak predictor, assume a high attributable risk because of its high prevalence.

Most of the factors included in the risk assessment indexes have been known to be associated with preterm birth and low birth weight for at least 20 years. Many factors, such as age, race, and socioeconomic status, do not cause preterm birth directly, and the mediating factors that lead to actual preterm delivery are poorly

Table I. Risk scoring indexes designed to predict preterm birth

Study and yr	*Adverse outcome measured*	*Screening system*	*Time of screening*	*Population*
Nesbitt and Aubry, 1969	PTB 21-37 wk	Own system designed to predict a variety of adverse pregnancy outcomes	First prenatal visit	969 clinic patients, N. Y. State, two thirds white, one third black
Kaminski et al., 1973	PTB <36 wk	PPRS	First trimester	Married multiparous French women with singleton pregnancies
Akhtar and Sehgal, 1980	PTB 21-36 wk	Own system designed to predict a variety of adverse pregnancy outcomes	Initial clinic visit updated at delivery	Rural, medically indigent, primarily white women in W. Va.
Creasy et al., 1980	Spontaneous PTB 20-37 wk	Modified PPRS	Initial screen at first visit, rescreened 26-28 wk	New Zealand hospital population 28% primigravid
Herron et al., 1982	PTL	Modified PPRS	Initial and follow-up screen 26-28 wk	Predominantly white middle-class in San Francisco
Main et al., 1987	All PTB 30-37 wk Spontaneous PTB 20-37 wk	Modified PPRS	Single screen <18 wk	Black Inner City women in Philadelphia
Ross et al., 1986	Spontaneous PTB 20-37 wk	Simplified problem-oriented perinatal risk assessment system	Retrospective review	Women in Los Angeles, predominantly Hispanic

PPRS, Papiernik prematurity risk score; PTB, preterm birth; PTL, preterm labor.

understood. Furthermore, most of the risks, including the demographic factors previously cited, uterine malformations, multiple gestation, and prior medical and obstetric history, cannot be changed by the person at risk or by health care providers.

Another limitation of these risk assessment tools is inherent in screening tools in general. High sensitivity is desired when the outcome predicted is very serious but is usually accompanied by lowered specificity and lowered positive predictive values. The incorrect identification of women with normal outcomes as high risk increases the emotional burden for these women and greatly increases the cost of additional medical interventions. The positive predictive value also depends on the prevalence of the adverse outcome in the population studied. In populations in which the outcome is common, the true-positive rate should increase, and the false-positive rate should be lower. False positives are more common when the outcome occurs infrequently, as observed by Herron et al.[2, 5] when they applied the scoring index to a group of predominantly white, middle-class women in San Francisco, a population with a preterm labor rate of 4.7%. In this prematurity prevention study, the sensitivity and specificity of the screening tool were only slightly less than that achieved in New Zealand. However, the positive predictive value was considerably lower. Only one of every six women identified as high risk experienced preterm labor.

We recently applied the same scoring system once to a group of 391 black, inner city women registered for prenatal care before 18 weeks' gestation. These women, who have a high baseline preterm delivery rate, received no special intervention to prevent preterm birth and were not aware of their risk status.[6] In this sample, the scoring system failed to distinguish effectively women who delivered before term from those who delivered at term. Women scoring ≥10 points (that is, high risk) had an 18% preterm delivery rate, whereas those scoring <10 points had a 14% preterm delivery rate. The sensitivity and specificity were similar for multiparous and primiparous women (28% versus 23% and 78% versus 82%, respectively). Adjusting the cut-off score and reweighting factors with discriminant analysis did not improve the predictive value of the scoring system. Analysis of an additional 60 social, demographic, and medical factors also failed to produce a useful risk assessment tool.

How can we explain the very different use of a scoring system in one population compared with another? First, the most predictive risk factors may be more prevalent in one population. Second, the scoring system may achieve much of its sensitivity from distinguishing between socioeconomic groups, a factor not considered in our universally low-income group. Alternatively, the significance of a particular risk factor may vary among populations. It is of interest that Rush[8] and co-workers found that the history of a prior low birth weight delivery was a much stronger predictor of repeat low birth

Table II. Accuracy of risk scoring systems

Study	PTB rate (%)	% assigned			Predictive value			
		High	Medium	Low	Sensitivity	Specificity	PV+	PV−
Nesbitt and Aubry, 1969	5.6	30	39	31	43*	71*	9*	96*
Kaminski et al., 1973	8.1	16	22	62	30‡	68‡	17‡	97‡
Akhtar and Sehgal, 1980	6.0	12	14	74	32*	90*	17*	96*
					49†	77†	13†	96†
Creasy et al., 1980	6.1	9‖	19‖	72‖	44*‖	93*‖	29*‖	97*‖
	6.1	13¶	19¶	68¶	64*¶	90*¶	30*¶	98*¶
	6.2	15#	17#	68#	77*#	90*#	33*#	99*#
	5.8	9**	25**	66**	31***	93***	21***	96***
Herron et al., 1982	2.4 (4.7 PTL)	15	—	85	56	87	18	97
Main et al., 1987	14.8††	36‖	—	64‖	26††	80††	18††	86††
	11.0‡‡	36‖	—	64‖	28‡‡	80‡‡	15‡‡	90‡‡
Ross et al., 1986	6.4	33	—	67	56	68	10	96

PTB, Preterm birth; PTL, preterm labor; PV+, positive predictive value; PV−, negative predictive value.
*Based on comparison of high-risk group versus medium- plus low-risk groups.
†Based on comparison of high- plus medium-risk groups versus low-risk group.
‡Based on comparison of high- versus low-risk groups with medium-risk group excluded from analysis.
§PTD rate lowered because of intervention program.
‖Single early screen only.
¶Includes repeat screening at 26 to 28 weeks.
#Rescreened multigravidas only.
**Rescreened primigravidas only.
††Predicting all PTB.
‡‡Predicting spontaneous PTB.

weight deliveries in white as opposed to black women. This discrepancy suggested to these authors that "with lower social status, fewer of the poor outcomes are caused by inherent physiological problems and more by adverse environmental conditions which would be less likely to be held constant between pregnancies." In addition, the relative contribution of the proximate causes for spontaneous preterm birth, namely, preterm labor and premature rupture of the membranes, may vary. This was recently well demonstrated by Meis et al.[9] Using two samples of approximately 1500 women each, one group receiving public medical assistance and one group with private insurance, they showed significant differences in proximate factors. In the public assistance group, 34% of preterm births were caused by preterm labor, 46% by premature rupture of the membranes, and 20% by medical complications. In contrast, in the private insurance group, 55% of preterm births resulted from preterm labor, 27% from premature rupture of the membranes, and 18% from medical complications. Specific risk factors may more strongly predict preterm labor rather than premature rupture of the membranes, although we were unable to demonstrate this in our relatively small sample. Finally, Creasy et al.[4] and Herron et al.[5] found that rescoring women in the second trimester, including specific pregnancy complications such as multiple gestation, bleeding, and cervical dilation, improved sensitivity.

Newer approaches to risk assessment

It has become apparent that three more specific approaches may be useful. First, cervical examinations and intermittent monitoring for contractions may more effectively identify women at risk for preterm labor. Second, a few risk factors appear sufficiently predictive to warrant more intensive evaluation during pregnancy, including daily medical contact and/or monitoring for uterine contractions. Third, some of the behavioral risk factors associated with preterm birth may be modified in pregnancy with improved outcome.

Risk assessment by cervical examination and assessment of uterine contractility. The association of cervical dilation in the late second and third trimesters with preterm labor has been emphasized by Papiernik and other European investigators. In a series of 4330 women examined at least once before 37 weeks' gestation, the relative risk for preterm delivery was approximately four times greater if the internal os was opened 1 cm or more.[10] Almost one quarter of the 4.4% of this population found to have cervical dilation between 25 and 28 weeks' gestation experienced preterm birth. This study was undertaken as part of a complex and successful intervention program to prevent preterm births. Therefore one can speculate that even higher rates of preterm births might have occurred in women with cervical change had interventions not been undertaken. Indeed, in a smaller, blinded, noninter-

vention study, Leveno et al.[11] studied the predictive value of a single cervical examination between 26 and 30 weeks' gestation. Cervical dilation of at least 2 cm was observed in 8% of the 185 women studied. Four of the seven low birth weight deliveries occurred in these 15 women.

Detection of increased uterine activity may best identify women with higher risks of preterm labor. We[12] monitored 148 black Inner City women for 1 hour on a weekly to biweekly basis between 27 and 34 weeks' gestation using a portable tocodynamometer. The study was completely blinded. All women had singleton pregnancies and had no history of preterm labor or delivery. The use of ⩾6 contractions per hour on at least one occasion between 28 and 32 weeks' gestation as a marker for excessive uterine activity would have identified 76% of the women who experienced preterm labor. Women so identified as at high risk had a 27% chance of developing preterm labor.

Daily home monitoring of uterine activity. An increased frequency of uterine contractions as assessed by daily home tocodynamometry appears to distinguish between high-risk women who develop preterm labor and those who do not.[13] Women included in studies that use ambulatory home monitoring have had very specific risks such as multiple gestation, uterine malformation, and repetitive preterm birth. At least one third of women so selected will develop preterm labor. In a recent randomized controlled study of very high-risk patients, Morrison et al.[14] were able to diagnose preterm labor earlier and significantly prolong gestation in 34 women monitored daily at home. The 33 control women were taught to identify the early signs of preterm labor and were contacted by telephone twice weekly by a nurse to assess their status. Two thirds of the women in each group experienced preterm labor. Although very exciting, such an intensive approach should be limited currently to women with select specific risks for preterm labor or women who have already experienced preterm labor in the current pregnancy. Further research is needed to confirm the efficacy of this approach in other high-risk patients and identify the components of this monitoring system that are most important.

Modification of behavioral risk factors for preterm birth. Our proposals are based on another approach, the aim of which is to define predictive factors which *can be modified during pregnancy*, or which lead to preventive action. Our hypothesis is that if positive and lasting changes are made in the habits of pregnant women, this can lead to a reduction in preterm birth rates *(emphasis added).*

E. Papiernik
Clinics in Obstetrics and Gynaecology
(London) (1984;11:815.)

At least five behavioral risk factors which may be associated with preterm birth could be modified in pregnancy. They include smoking, poor weight gain during pregnancy, absent or inadequate prenatal care, heavy physical labor, and psychological stress. Relatively few studies, however, have been designed to evaluate the effects of modifying these behaviors during pregnancy.

Interestingly, Papiernik et al.[15] attribute most of the overall reduction in preterm births in Haguenau, France, to reduction in preterm births among low- and medium-risk women rather than among women with classic risk factors such as prior preterm births or bleeding during pregnancy. The main intervention for these women was a careful analysis of their physical activity and recommendations to decrease hard physical labor. The French government supported compensation during periods of unemployment. However, most other studies have not associated preterm birth with paid employment or physical activity. Thus more research is needed that links excessive uterine contractility to heavy physical activity in low-risk women before widespread reduction in physical activity during pregnancy can be recommended.

Sexton and Hebel[16] have demonstrated in a prospective, randomized, and controlled trial that pregnant smokers who participated in an individualized smoking cessation project more often stopped smoking and as a group were delivered of significantly heavier babies than were control patients. These impressive results were obtained even though only one out of three women who still smoked at the time of enrollment into the study quit smoking during pregnancy. There was no effect on gestational age. Further research is needed regarding effective means to promote smoking cessation, reduction of other substance abuse, improved nutritional practices, and more complete health care access. Better understanding of attitudes toward pregnancy, health care beliefs, and daily habits and stresses of pregnant women with diverse backgrounds will facilitate such intervention programs.

Comment

Traditional risk scoring indexes may be of benefit in select populations. Persons using such scoring systems, however, should demonstrate that they can effectively identify high-risk women within their population. One cannot concurrently test a scoring system and an intervention program in the same group of patients. Effective interventions may mask the predictive value of the scoring system.

Careful assessment of uterine contractility and cervical change in unselected women may improve both our ability to identify women at high risk for preterm birth and our understanding of the mechanisms lead-

ing to preterm labor and premature rupture of the membranes. Accurate daily ambulatory tocodynamometry appears to have clinical value in very high-risk patients.

Emphasis on risk factors that can be modified during pregnancy may have the greatest success in decreasing the incidence of preterm birth in women without classic medical or historical risk factors. It is clear that programs to modify women's habits during pregnancy are difficult and take time to institute.[15] More studies designed to modify behavior by means of clearly defined, limited interventions are needed.

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Prophylactic antibiotics for cesarean delivery: A simple cost-effective strategy for prevention of postoperative morbidity

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Postoperative endomyometritis is the most common complication associated with cesarean delivery. The frequency of endomyometritis varies from 5% to 85% (mean 35% to 40%), depending on the socioeconomic characteristics of the patient population and the indication for abdominal delivery. In women undergoing cesarean section after extended duration of labor and ruptured membranes, the use of prophylactic antibiotics has been consistently effective in decreasing the frequency of endomyometritis. A single dose of antibiotic administered after the umbilical cord is clamped provides a degree of prophylaxis comparable to that achieved with two- and three-dose regimens. There is no evidence that the newer extended-spectrum cephalosporins or penicillins are better for prophylaxis than ampicillin or a first-generation cephalosporin. Administration of antibiotics by intraoperative irrigation is an acceptable, but not superior, alternative to systemic antibiotic administration. Prophylactic antibiotics alter the vaginal flora after operation, but to date harmful clinical effects have not occurred as a result of such alteration. (Am J Obstet Gynecol 1987;157:794-8.)

Key words: Cesarean delivery, antibiotic prophylaxis

The most common complication associated with cesarean delivery is endomyometritis. The frequency of infection varies from 5% to 85%, with a mean incidence of 35% to 40% in most studies.[1,2] In general, the highest incidence of infection occurs in indigent women having cesarean delivery after extended duration of labor and ruptured membranes. Many investigations have demonstrated that prophylactic antibiotics are effective in decreasing the frequency of post–cesarean section endomyometritis in women having unscheduled abdominal delivery. However, some controversy still exists about which antibiotics should be used for prophylaxis, duration of use and in what manner they should be administered. The purpose of this article is to present a simple, cost-effective plan for utilization of prophylactic antibiotics in women having cesarean delivery.

Criteria for use of prophylactic antibiotics

Three clinical criteria should be fulfilled to justify perioperative use of prophylactic antibiotics. The surgical procedure, by necessity, must be performed through a contaminated operative field. There must be a high incidence of postoperative infection, that is, exceeding 15% to 20%, in the absence of prophylaxis. Finally, there must be serious sequelae that may result from the primary infection.

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Unscheduled cesarean delivery clearly fulfills these criteria. The operation inevitably is associated with considerable bacterial contamination of the endometrial and peritoneal cavity. If some form of prophylaxis is not administered, the incidence of post–cesarean section endomyometritis is unacceptably high in most patient populations, averaging 35% to 40%.[2] In patients who have endomyometritis, potentially life-threatening sequelae such as septic shock, pelvic abscess, and septic pelvic vein thrombophlebitis can occur.

Mechanism of action of prophylactic antibiotics

Prophylactic antibiotics appear to act in two principal ways. By destroying some bacteria and slowing the growth of others, they directly decrease the size of the bacterial inoculum at the surgical site. Prophylactic antibiotics also alter the characteristics of the serosanguineous fluid that collects in the pelvic cavity after operation, rendering it less suitable to support the growth of microorganisms. Other possible mechanisms of action include interference with the production of bacterial proteases and interference with attachment of bacteria to mucosal surfaces. In addition, antibiotics may, in a way that is not completely understood, enhance the host's phagocytic capacity.[3]

Burke[3] has demonstrated that the timing of antibiotic delivery to injured tissue is of critical importance in determining the efficacy of prophylaxis. The greatest therapeutic effect occurs when antibiotics are administered just before, or coincident with, the time when

Table I. Limited- versus extended-spectrum agents as prophylaxis for cesarean delivery

Author	*No. of patients*	*Drugs**	*Endometritis (%)*	*p Value*
Louie et al.[5] (1982)	70	Cefazolin	4.3	NS
	60	Ampicillin	3.3	
	58	Cefotaxime	6.9	
Stiver et al.[6] (1983)	119	Cefazolin	2.5	NS
	124	Cefoxitin	4.0	
Rayburn et al.[7] (1985)	124	Cefazolin	3.2	NS
	130	Moxalactam	7.7	

*Three-dose regimens.

maximal bacterial contamination and tissue trauma occur.

Objectives in the use of prophylactic antibiotics

One major objective in the use of prophylactic antibiotics is reduction in the incidence of endomyometritis. This infection causes the patient considerable discomfort and usually results in increased duration and expense of hospitalization. In addition, it is the usual precursor for such serious complications as pelvic abscess, septic shock, and septic pelvic vein thrombophlebitis. A second objective in the use of prophylaxis is reduction in the incidence of major wound infections. Such disorders usually require a second surgical procedure to open and debride the incision and may result in dehiscence and evisceration. For these reasons, wound infection is even more likely than endomyometritis to result in marked prolongation of the patient's hospitalization. The third theoretical objective in the use of prophylaxis is decrease in the duration and expense of hospitalization. The final objective is prevention of the rare, life-threatening complications of operative site infection.

Results of clinical trials of systemic prophylaxis

To date more than 25 prospective, randomized, placebo-controlled trials of systemic prophylaxis for cesarean delivery have been published.* The most important findings of these studies are summarized below.

1. Virtually without exception, all studies have demonstrated a significant decrease in the frequency of endomyometritis in women having unscheduled abdominal delivery after extended duration of labor and ruptured membranes. The reduction in the incidence of endomyometritis has averaged 50% to 60% in most studies.[2] These data are so convincing that I believe it is not ethical to use placebo controls in future studies of prophylaxis in these high-risk patients.

2. Most investigations have not shown a statistically significant decrease in endomyometritis in women undergoing elective, scheduled abdominal delivery. However, there may be some patient populations in which the underlying frequency of infection is high enough that prophylaxis does have a beneficial effect in reducing the incidence of postoperative morbidity and decreasing the expense of hospitalization.[4]

3. The drugs most frequently used for prophylaxis have been the cephalosporins. Although it is widespread practice in the United States to utilize the newer extended-spectrum cephalosporins for prophylaxis, there is no evidence that these agents are better for this purpose than ampicillin or a first-generation cephalosporin. To date three authors[5-7] specifically have compared limited-spectrum agents to extended-spectrum antibiotics for prophylaxis. In these trials, 312 patients received an extended-spectrum agent and 373 women received a more limited-spectrum agent (Table I). None of the individual trials, nor all considered collectively, has been able to show that the more expensive, extended-spectrum drugs are more effective in decreasing the incidence of postoperative endomyometritis.

4. Most investigators have used a three-dose regimen for prophylaxis. However, there now are at least seven reports demonstrating that a single dose of antibiotic is comparable in efficacy to multidose regimens. Three of these investigations[8-10] compared single doses of antibiotic to placebo. In each instance, patients who received antibiotics fared better than placebo controls and as well as historical controls in other studies that used conventional three-dose regimens for prophylaxis. Three additional studies[11-14] compared a single dose of one drug to three doses of the same drug or another agent. There were no significant differences in treatment effects between the groups.

5. Three investigators[15-17] specifically sought to determine whether an extended course of antibiotics was more effective than limited perioperative prophylaxis in decreasing the incidence of post–cesarean section infection. Elliott et al.[15] demonstrated that a 3-day course of ampicillin was more effective than a three-dose regimen in decreasing the incidence of febrile morbidity and endomyometritis and in shortening the

*A complete list of references is available on request from the author.

duration of hospitalization. However, D'Angelo and Sokol,[16] were unable to confirm a significant difference in the combined incidence of wound infection and endomyometritis when a 5-day course of treatment with cefazolin plus cephalexin was compared with a four-dose course of cefazolin. DePalma et al.[17] studied two drug regimens (cefamandole and penicillin plus gentamicin) and were unable to confirm an advantage for extended early therapy versus limited perioperative prophylaxis. This latter study is noteworthy because the authors' patients were at exceptionally high risk of post–cesarean section infection as a result of their low socioeconomic status and extended duration of labor and ruptured membranes.

At the present time it is not possible to identify precisely that subset of women who are at such high risk of infection that they need early treatment with therapeutic antibiotics rather than simple prophylaxis.[18] Until a laboratory or clinical test with high sensitivity and predictive value is available, extended prophylactic or "early treatment" regimens cannot be recommended.

6. For most surgical procedures, it is desirable to administer prophylactic antibiotics preoperatively before tissue injury and bacterial contamination occur. If this policy were implemented for cesarean delivery, the neonate would be exposed to antibiotic before birth. In theory, even this limited exposure might interfere with the ability of the pediatrician to evaluate the newborn for sepsis. Fortunately, multiple reports now have confirmed that delay in administration of antibiotics until after delivery of the fetus does not diminish the effectiveness of prophylaxis.

7. Most investigators have been unable to demonstrate that antibiotic prophylaxis decreases the incidence of abdominal wound infection. In the studies in which a positive effect was confirmed, the incidence of wound infection in the control population was high, ranging from 7.4% to 16.1%. In the studies that showed no reduction in wound infection, the incidence of infection in the control population was lower, ranging from 0% to 7%. With such a low frequency of infection in the control groups, many of the studies with negative findings may not have had a sufficient sample size to detect a beneficial treatment effect.

8. Use of prophylactic antibiotics has not eliminated completely the occurrence of serious sequelae of postcesarean endomyometritis. Despite receiving prophylaxis, some patients still have septicemia, pelvic abscess, or septic pelvic thrombophlebitis. However, the incidence of such complications is low, even in patients receiving no prophylaxis.[1,2]

9. At present, no serious maternal side effects have occurred as a result of use of antimicrobial prophylaxis for cesarean delivery. There are case reports, however, of at least two deaths from anaphylaxis in patients who received intraoperative injections of cephalothin during nonobstetric surgical procedures.[19] Therefore, the possibility of untoward side effects always must be considered when a decision is made to use prophylactic antibiotics.

10. Several recent investigations[20,21] have shown that changes in the vaginal flora occur in women who receive prophylactic antibiotics at the time of cesarean delivery. The alterations in flora depend on the specific drug selected for prophylaxis and the length of time it is administered.

The clinical significance of these changes in the flora of the lower genital tract is not entirely clear. No investigator yet has documented an increased incidence of more serious complications in women who become infected despite receiving prophylaxis. However, of particular concern is the increased isolation of enterococci in women receiving prophylactic cephalosporins. Many antibiotics used singly or in combination for treatment of postcesarean infection do not provide effective coverage against enterococci. Therefore, if prophylactic antibiotics are to be used routinely on an obstetric service, careful surveillance must be maintained to detect unusual changes in antimicrobial susceptibilities.

There are three principal components of an effective surveillance system. First, genital tract cultures should be performed in all patients who become infected despite receiving prophylaxis. The antibiotic susceptibility patterns of bacteria isolated from these patients should be evaluated to determine whether selection of resistant microorganisms is occurring. Second, the clinical course of these women should be monitored to determine whether they are experiencing a suboptimal response to the usual antibiotic regimens selected for treatment of postoperative infection. Finally, the hospital laboratory should conduct a periodic review of the susceptibility patterns of commonly isolated microorganisms to detect gradual changes in antimicrobial resistance throughout the hospital.

Intraoperative antibiotic irrigation

Intraoperative antibiotic irrigation appears to exert its primary effect by providing a high concentration of antibiotic directly to the site of injured tissue and the site of maximum bacterial contamination. Systemic absorption of antibiotic occurs with topical irrigation, however, especially when both intrauterine and intraperitoneal irrigation is performed.[21] Serum concentrations of antibiotics, in fact, may exceed the minimal inhibitory concentrations for many genital tract pathogens. Therefore, the mechanism of action of intraoperative irrigation appears to be due, at least in part, to systemic absorption of antibiotic and not exclusively to a local effect on the endometrium.

There now are more than 10 studies of intraoperative irrigation for cesarean delivery.* The principal findings of these investigations are summarized below.

1. Most of the studies have shown that antibiotic irrigation is superior to placebo or saline irrigation.

2. Unlike systemic prophylaxis, not all antibiotics have been equally effective when administered by irrigation. The observed differences in treatment effect appear to be partially due to differences in irrigation techniques. For example, there are differences between the volume of diluent, and hence the concentration of antibiotic, used in different studies. Some authors have irrigated both the uterine and peritoneal cavity; others have irrigated only the former. Some investigators have allowed the irrigant solution to remain in the uterus and peritoneal cavity, whereas others have immediately aspirated the solution. Differences in the pharmacokinetic properties of the drugs, particularly in half-life of the antibiotic in serum and tissue, also may be important in explaining the varied results of these studies. On the basis of information presently available, cefamandole appears to be the agent with the most thoroughly documented evidence of efficacy.

3. Most of the studies that have compared intraoperative irrigation to systemic prophylaxis have used three doses for systemic administration and one dose for irrigation. In a comparison of this nature, there would appear to be a distinct cost advantage for irrigation versus systemic administration. However, recent reports[8-14] have shown that a single dose of systemic prophylaxis can be just as effective and economical as a single dose of drug administered by irrigation.

In view of these latter findings, the principal argument in favor of irrigation is that it minimizes systemic absorption of antibiotic and hopefully reduces the risk of an untoward drug effect. Conversely, the major advantage of systemic prophylaxis is that it is a simpler and less time-consuming method of antibiotic administration.

4. Use of systemic antibiotics in conjunction with intraoperative irrigation does not enhance the effectiveness of prophylaxis.[23] Therefore, there is no justification for combining both methods.

Cost considerations

As outlined, the use of prophylactic antibiotics clearly is effective in decreasing the frequency of postcesarean endomyometritis. This certainly is an important accomplishment from the perspective of the individual patient. In addition, it is a significant achievement from the perspective of the entire health care industry because of issues related to hospitalization cost.

*A complete list of references is available on request from the author.

Consider a hypothetical cohort of 100 high-risk women undergoing cesarean delivery. Let us assume that, in the absence of prophylaxis, endomyometritis develops in 40 patients. It would be reasonable to expect that prophylaxis would decrease the incidence of infection by 50%. Therefore, failure to use prophylaxis results in 20 unnecessary infections.

Let us now consider the cost of treating these 20 patients. The usual parenteral antibiotic regimens used at this institution for treatment of endomyometritis are mezlocillin (3 to 4 gm every 6 hours) or clindamycin (900 mg every 8 hours) plus gentamicin (1.5 mg/kg every 8 hours). Treatment usually is required for a minimum of 4 days. The total wholesale cost for either of these antibiotic regimens is approximately $140. Thus the total cost of treating 20 infected patients would be $2800. Infected patients typically remain hospitalized for 2 days longer than uninfected women, and the hospital's unit charge per patient is $441 per day. Therefore, the added cost for extended hospitalization of the 20 patients is $17,640. These, of course, are minimum estimates because they do not include any added pharmacy charges or incidental expenses that result when a patient has a protracted febrile course.

Balanced against these charges is the cost of the antibiotic selected for prophylaxis. As outlined earlier, the most reasonable choice for prophylaxis would appear to be a single 2 gm dose of a first-generation cephalosporin, such as cefazolin or ampicillin. The costs of these drugs at this hospital are $6.00 and $3.00, respectively. The total cost of administering 100 doses of prophylaxis would be $600 and $300, respectively. Therefore, the net cost savings resulting from use of either of these prophylactic regimens is approximately $17,000 for every 100 surgical procedures. Even if two-dose courses of prophylaxis were used, the savings in costs exceeds $16,000 per 100 procedures. At this institution, approximately 200 unscheduled cesarean deliveries are performed each year, thereby resulting in a minimal savings of at least $32,000 per year.

In patient populations with a lower baseline frequency of infection than noted herein, the savings in cost will be less. Routine use of multidose regimens of the more expensive extended-spectrum agents also will reduce the favorable economic impact of prophylaxis.

Conclusions

Prophylaxis should be used in all women who are in labor or who have ruptured membranes.

Prophylaxis also should be considered in women having elective repeat cesarean delivery if the baseline frequency of endomyometritis or wound infection is unusually high. If the combined incidence of these two infections is in the range of 15% to 20%, use of pro-

phylaxis may have a beneficial effect in reducing morbidity and hospital expense.

For systemic prophylaxis, a single dose of ampicillin (2 gm) or a first-generation cephalosporin such as cefazolin (2 gm) should be administered after the infant is delivered. A second dose of antibiotic should be used only if the operation is prolonged for more than approximately 90 minutes. The rationale for administration of a second dose of antibiotic is that extended operations are likely to be associated with greater bacterial contamination and tissue injury.[3] The second dose of drug should be administered 3 to 4 hours after the first dose.

Use of extended-spectrum cephalosporins or penicillins for prophylaxis should be avoided unless future investigations are able to confirm a decided advantage for the more expensive agents. Indiscriminate use of these drugs for prophylaxis may limit their usefulness in treatment of established infections.

Intraoperative irrigation is an acceptable, but not superior, alternative to systemic prophylaxis. Combining irrigation with systemic administration does not enhance the effectiveness of prophylaxis. Cefamandole is the agent that has been the most extensively tested for irrigation.

When antibiotic prophylaxis is used routinely on an obstetric service, the antimicrobial susceptibility patterns of bacteria isolated from infected patients should be analyzed carefully to detect the emergence of drug-resistant microorganisms and the occurrence of serious infections.

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Presidential address of Association of Professors of Gynecology and Obstetrics

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It is a significant honor to be recognized by one's peers and to be given the opportunity to lead an organization whose primary goal is the perpetuation of our discipline. It is a particular honor to serve in that role in the twenty-fifth anniversary year of the Association of Professors of Gynecology and Obstetrics, which also represents for me a twenty-fifth anniversary in academic medicine. The goals of my presidential address are limited and simple: to express some aspects of personal philosophy; to summarize the problems we face in medicine, in academic careers, and in our discipline; to review the changes in the Association of Professors of Gynecology and Obstetrics and our specialty in the past 25 years; and to provide some inspirational thoughts about the future.

Persons in my position have a problem. While we are medical teachers in that we have taught students for 25 or more years, we are not experts in education. Although we cannot explain it in educational terms, we have developed an instinct for those approaches in medical education that work and those that do not. It is this instinct that makes some of us react so negatively to the suggestions of those who work in the theory rather than the practice of medical education. For example, the concept that in an ideal world, medical education would be a totally integrated and interdisciplinary experience is one that I do not find very convincing. In my experience, integrated teaching is difficult to plan and to mount, expensive, and extremely time-consuming for the organizers, and it is confusing for the students who have to grapple simultaneously with the different approaches of a variety of persons and disciplines. Of more importance, it is potentially harmful for departments, since it eliminates their strongest stimulus—the responsibility for their own teaching.

Another currently popular concept is that "students should be taught principles rather than facts".[1] The truth is that both are needed. Principles are pretty dull unless the facts that they tie together are known. It is not exciting to have a solution without a problem. Principles are really exciting only when they help us fit together the various bits of knowledge and experience that float about in our heads and make the whole into an attractive story.

We are also told that students should not be taught; it makes them mentally lazy. They should discover their own areas of ignorance and respond appropriately. Perhaps the reason that I find this so hard to accept is my enormous sense of debt and gratitude to the excellent faculty who shared with me the wisdom they had built up through years of experience. I do not think it made me mentally lazy; in fact, I think it opened my mind to all forms of curiosity and adventure. I really enjoy listening to someone who knows his or her subject intimately—and loves it. That teacher can give a particular insight as to how the mind works and what the subject is really about.

Let me now review some of the problems that face us in 1987. After World War II biomedical science was able to achieve major advances in the relief of suffering, the rehabilitation of injuries and the prevention of disease and premature death. This success led to an increased demand for medical services and resulted in a perceived shortage of physician manpower. By 1962, the first year of the Association of Professors of Gynecology and Obstetrics, this country was prepared to

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Table I. Presentations at meetings of the Association of Professors of Gynecology and Obstetrics

1967 "Medicare and undergraduate education," Dr. John Van S. Maeck
1969 "The consumer in medicine," John Mills, Ph.D., Chancellor, Case Western Reserve University
1970 "Intradisciplinary specialization," Drs. F. P. Zuspan, D. A. Barron, J. G. Moore, and W. L. Herrmann
1972 "HMO's," Drs. P. D. Bruns and J. R. Willson
1973 "Medicine and government in Canada," Dr. R. P. Beck (University of Alberta)
1974 "Faculty funding," Drs. K. E. Krantz and W. H. Pearse
1976 "The education of women in OB/GYN," Drs. E. R. Carrington and M. J. Gray
1977 "Medico-legal problems," Dr. K. P. Russell

mobilize for a substantial expansion of its capacity to train health professionals. Academic medical centers produced and applied much of the new biomedical knowledge and helped, in a major way, to fulfill society's medical commitments to its indigent members. In 1987 it appears that society sees a physician glut and has recognized the limits of its ability, or at least its willingness, to accord a protective status to these institutions. Efforts to curb escalating expenditures for the care of elderly persons and indigent persons have resulted in new payment systems that are designed to foster price competition and to eliminate hidden subsidies for teaching institutions.[2]

The primary environmental change affecting medical education is an economic one. Teaching hospitals are motivated to cut expenses, increase efficiency, and be more businesslike. Because of decreasing support for research and reduced funding in support of medical education, medical schools are encouraging faculty members to supplement their salaries by an increase in the number of patients they treat.

Large-scale purchasers of medical services are seeking to contract with hospitals and physician groups that will provide acceptable quality of services at the lowest cost. These purchasers do not believe that educational costs are their responsibility. The implications for the academic medical centers are ominous inasmuch as they also provide a disproportionately large share of the most sophisticated, intensive, and expensive hospital services. In particular this care is provided to medically indigent persons. Six percent of these hospitals provided 47% of the charity care charges and incurred 35% of the bad debts in the country.[2] Obstetric care alone accounted for nearly 25% of all uncompensated hospital charges. In 1983, this amount was $1.7 billion. Regionalization of perinatal care (at least as it involves the medically insured patient) is more difficult because hospitals are encouraged to compete rather than cooperate.

Teaching services are having difficulty in the maintenance of the patient base necessary for their mission inasmuch as well-trained alumni now provide, in community hospitals, specialized services that were available earlier only at academic medical centers. What we, in the academic medical center, produce is a valued commodity, with no proprietary rights to it. It is different from inventing an item at Bell Laboratories, which the company markets and benefits are reaped for 17 years.[3] Our product, the student, resident, fellow, or the results of research, is rapidly translated to patient care in a variety of settings, often to the detriment of the medical center.

In addition, the experience students and residents receive on a tertiary in-patient service is composed of chronic and complex care and is generally not related to situations that they will face in practice. Increasingly, with same-day admissions and early discharge, they do not get to see the entire span of medical events. The obvious solution, the development of large affiliated programs with a community hospital base, goes a long way toward solving the numbers problem in student and resident training, but it also disperses and dilutes the educational effort and the continuity of care that is possible in a single-unit health center.

And what of the product of our efforts? Students and residents have always been anxious about beginning their medical careers, but these concerns focused more on quality-of-living issues and practice environment. These have been replaced by some very real issues, such as fears of a surplus of physicians, the prospect of competing with professional colleagues and former classmates, the decline of the traditional, solo fee-for-service medical practice, the loss of autonomy from health care institutions, government, and third-party payors, and the impact of technological change.[4]

Although there has been no perceptible decline in the quality of the credentials presented by applicants entering medical school, the number of applicants has decreased steadily since 1974 and a decline in the quality of the applicant pool should be anticipated.

At times like this all of us need to step back from these problems and look for some perspective in dealing with them on the basis of where we have been. I would like to look at "APGO at 25" to remind all of us of the enormous changes that have occurred since the founding of this society.

Table II. Changes in field of health care

	Then	*Now*
National health expenditures	$26.5 billion	$381.2 billion
Percentage research-development	4.8%	3.0%
Total number of physicians	274,000	486,000
Obstetrician-gynecologists	14,000	34,000
U.S. medical schools	86	127
Applicants	15,740	29,000 (1975 peak 42,000)
Women applicants	8.1%	35.2%
Graduates	7,480	16,875
Education expenditures	$436 million	$9,000 million
Federal funding	48.1%	23.5%
Physician's services for funding	6.0%	35.9%
Tuition		
State (mean)	$498	$3,959
Private (mean)	$1,050	$13,105
Full-time clinical faculty	8,750	45,300
Postgraduate salary (1 yr)	$2,465	$21,800
Postgraduate women (1 yr)	8%	51%
Malpractice premium (Washington, D.C.)	$640	$58,000

The first meeting of the Association of Professors of Gynecology and Obstetrics was held at the Pick-Congress Hotel in Chicago in 1962, with 175 people in attendance under the leadership of the first president, Roy Holly, M.D. The first five programs focused entirely on curriculum, evaluation, and pedagogy in undergraduate and graduate education. Inevitably, thereafter, the problems of the real world intruded into the program (Table I). These problems have dramatically changed the face of the entire educational enterprise during the past 25 years. Some of these changes are depicted in Table II. The focal points include the real as well as inflationary increases in both total national health costs and the costs of medical education; the increase in the number of medical schools, medical students, clinical faculty, practicing physicians, and specialists in obstetrics and gynecology; the women's movement, with its major impact on medical education; and the steadily decreasing component of educational costs funded by the federal government.

From a historical perspective, it is also important to remember that many of these changes occurred in response to predicted needs that were perceived as largely unmet 25 years ago. The accuracy of our predictions was not high. "The Census Bureau projection of 6,400,000 births in 1985 bears specifically on the need for more maternity beds and more medical manpower in this specialty."[5] The actual number of births in 1985 was 3,747,000. There have been many changes in our discipline in this relatively short time span. "Since there is such a small chance of the survival of an infant under 1,000 gms., (less than 5%), all such fetuses should be classified as abortuses."[5] Also a word of caution about the excesses of operative delivery when the national cesarean section rate had climbed to 4.8%: "There was a time when the excellence of an obstetric service was judged by the scarcity of cesarean sections performed. There has been a tremendous shift in viewpoint regarding the validity of this criterion resulting in the current increased rates."[5] One needs only to reflect on the fact that none of the techniques, therapies, or clinical diagnoses depicted in Table III were clinically relevant 25 years ago.

Table III. Innovations and areas of concern in obstetrics-gynecology since 1962

Real-time ultrasound
Tocolytics
Legalized abortion
Laparoscopy
In vitro fertilization–embryo transfer
Gonadotropin-releasing hormone
Amniocentesis–chorionic villus sampling
Laser
Herpes
Pergonal
Cryosurgery
Acquired immune deficiency syndrome
β-subunit of human chorionic gonadotropin
Microsurgery
Danocrine
Surrogates
Fetal monitoring

Accurate assessments of the future for today's students do not exist because of the difficulty of predicting changes in health care utilization, technology, and patterns of care provision. The changing environment of medicine presents both threats and opportunities for young physicians. The very nature of technological progress makes the role of technology less certain. Some advances may reduce the demand for the services of a particular specialty if the technology can be used

by nonphysicians. Others may require more physician involvement because of the need for professional sophistication in their use.[4] Other advances transform the very nature of a specialty inasmuch as we are now seeing the so-called noninvasive cardiologic techniques impacting on the field of cardiovascular surgery. In turn, magnetic resonance imaging, by accurately visualizing coronary artery occlusions, could obviate catheterization skills learned in current cardiology fellowships. This is a form of technological vulnerability.

The demographics of health care are changing. Visits to the physician increase as the patient ages. Women, after the age of 17, consistently have a greater number of visits to the physician per year. In a population that is rapidly aging and one in which women will make up a decided majority of elderly persons, these trends will strongly influence the nature of our practices and how we train for the specialty. The changing structure of health care is already visible in a variety of "turf disputes" resulting in a decline of collegiality within the physician community.[6]

Pressure will increase to limit the growth in physician supply from both within and outside the profession. Reduction in the size of medical school classes, in the face of significant fixed costs, is not associated with a parallel decrease in the cost of education. Smaller class sizes will inevitably result in higher tuitions; this will further discourage economically disadvantaged students from applying for admission to medical school. Closing medical schools is a solution, but the list of volunteers is small. There will also be a move to reduce the number of graduate medical education training positions. This will intensify the competition for residents' positions.

Despite all these potentially adverse consequences to individual physicians, I believe that innovations in medical education, technology, and the organization of medical practice will continue to improve the ability of physicians to provide high-quality medical care to their patients. After years of change driven by biomedical discoveries emanating from the academic medical centers, there is a new site of innovation within the delivery system itself and those in the forefront are not academicians.[4] It is important that, as we translated what we have learned in the laboratory to the delivery system in the past, we must now take what is being learned in the marketplace and bring it back to the classroom.

Each physician must understand the changing horizon of medicine and be able to take whatever steps are necessary to remain professionally flexible. This capacity needs to be taught. The challenge lies with those of us who teach our specialty to demonstrate that flexibility and to identify and train the leaders for the future of medical practice from among the current medical students.

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A sonographic screening method for Down syndrome

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We describe a potential screening method for the second trimester detection of the fetus with Down syndrome by the use of standard sonographic biometry. Retrospective assessment of the biparietal diameter, femur length, and biparietal diameter/femur length ratio in 55 fetuses with Down syndrome and 544 control fetuses was performed at two medical centers. Adequate numbers of cases and controls were available to permit statistically significant comparisons between the groups at 15 to 23 weeks' gestation. Fetuses with Down syndrome displayed no statistically significant differences in the cephalic index or the biparietal diameter compared with controls at a given gestational age. Significant femur length shortening was observed, but the greatest statistical difference was noted for the biparietal diameter/femur length ratio. This ratio was found to decrease with gestational age in the normal population and was consistently elevated in the Down syndrome population (compared with control) throughout the second trimester. With a cutoff value of 1.5 SD above the normal population mean for the biparietal diameter/femur length ratio, 50% to 70% of fetuses with Down syndrome could be identified in 6% of the total population ($p < 0.0001$). This preliminary study suggests that the biparietal diameter/femur length ratio may prove superior to current Down syndrome screening methods and, as a third independent variable, may significantly enhance the sensitivity and specificity of combined maternal age/maternal serum α-fetoprotein screening modalities. (AM J OBSTET GYNECOL 1987;157:803-8.)

Key words: Down syndrome, sonography, biparietal diameter, femur length, screening methods

The antenatal detection of Down syndrome before 24 weeks' gestation remains a major epidemiologic challenge. The sensitivity of maternal age–based Down syndrome screening programs has decreased as a result of a progressive decline in births to women over 34 years of age. Presently, <20% of Down syndrome cases are associated with a maternal age >34 years.[1] The introduction of screening techniques for Down syndrome that use decreased maternal serum α-fetoprotein concentrations enables assessment of the 95% of pregnant women <35 years old who are responsible for 80% of Down syndrome fetuses. However, such programs are also associated with a low sensitivity (13% to 21%) and high false-positive rate (7% to 9%).[2, 3]

The increasing use of routine ultrasound in the second trimester has stimulated an interest in discovering a simple, readily reproducible sonographic method that identifies pregnancies at high risk for Down syndrome. Thus we performed a retrospective analysis of sonographically derived biometric differences between Down syndrome and normal fetuses at two prenatal diagnosis centers. A screening method exploiting the increased biparietal diameter/femur length ratio of Down syndrome fetuses is proposed.

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Material and methods

Control population. The ultrasound reports of all consecutive patients undergoing genetic amniocentesis at the Yale University Perinatal Unit who were between 15 and 23 weeks' gestation were reviewed for the period January 1, 1986, to October 1, 1986. Indications for amniocentesis included advanced maternal age (63%), abnormal maternal serum α-fetoprotein concentrations (34%), and a family history of genetic disorders (3%). Exclusion criteria included a discrepancy of >1.5 weeks between the menstrual age and gestational age derived from biparietal diameter and femur length measurements,[4, 5] the failure to record both biparietal diameter and femur length measurements, or the presence of anatomic or chromosomal anomalies. A total of 349 patients was available for analysis in the New Haven control population.

Utilizing similar exclusion criteria, the ultrasound reports of 195 consecutive patients undergoing genetic amniocentesis for advanced maternal age and low ma-

Table I. Comparisons between cases and controls at 16, 18, and 20 weeks' gestation for the New Haven population

Variable	*Gestational age (wk)*	*Group*	*No.*	*Mean*	*SD*	t *test*	*Wilcoxon test*
Gestational age	16-17	DS	13	16.4	0.38		
		NL	172	16.4	0.35	$p < 0.64$	$p < 0.63$
	17.5-18.5	DS	10	18.1	0.36		
		NL	79	18.1	0.39	$p < 0.56$	$p < 0.59$
	20-21	DS	10	20.3	0.40		
		NL	26	20.3	0.28	$p < 0.85$	$p < 0.42$
Cephalic index	16-17	DS	5	0.82	0.03		
		NL	104	0.80	0.04	$p < 0.14$	$p < 0.09$
	17.5-18.5	DS	5	0.79	0.04		
		NL	57	0.80	0.04	$p < 0.85$	$p < 0.84$
	20-21	DS	6	0.82	0.05		
		NL	19	0.82	0.04	$p < 0.84$	$p < 0.75$
Biparietal diameter	16-17	DS	13	3.51	0.21		
		NL	172	3.60	0.22	$p < 0.13$	$p < 0.24$
	17.5-18.5	DS	10	4.13	0.32		
		NL	79	4.04	0.23	$p < 0.28$	$p < 0.13$
	20-21	DS	10	4.77	0.38		
		NL	26	4.73	0.30	$p < 0.72$	$p < 0.53$
Femur length	16-17	DS	13	1.87	0.24		
		NL	172	2.13	0.21	$p < 0.0001$	$p < 0.0002$
	17.5-18.5	DS	10	2.33	0.26		
		NL	79	2.59	0.21	$p < 0.0007$	$p < 0.006$
	20-21	DS	10	2.99	0.23		
		NL	26	3.29	0.28	$p < 0.003$	$p < 0.003$
Biparietal diameter/femur length ratio	16-17	DS	13	1.90	0.18		
		NL	172	1.71	0.14	$p < 0.0001$	$p < 0.0002$
	17.5-18.5	DS	10	1.77	0.20		
		NL	79	1.57	0.11	$p < 0.0001$	$p < 0.002$
	20-21	DS	10	1.61	0.06		
		NL	26	1.44	0.09	$p < 0.0001$	$p < 0.0001$

DS = Down syndrome; NL = normal.

ternal serum α-fetoprotein concentration at the Diagnostic Ultrasound Associates in Boston were reviewed for the period August 1, 1986 to October 1, 1986. Gestational ages ranged from 15 to 21 weeks.

Down syndrome population. All consecutive karyotypic diagnoses of trisomy 21 made at the Yale University Cytogenetics Laboratory from January 1, 1984, through October 1, 1986, were ascertained. Karyotype sources included amniotic fluid derived from genetic amniocenteses (91%) and lymphocytes from neonates with Down syndrome stigmatas (9%). Those women bearing Down syndrome fetuses who had undergone second-trimester obstetric ultrasound examinations at the Yale Perinatal Unit before the diagnosis of Down syndrome were identified. Exclusion criteria consisted of intrauterine fetal death, hydrocephalus, inaccurate dating criteria, or a failure to record both biparietal diameter and femur length measurements. Ultrasound data were available in 35 patients from 15 to 23 weeks' gestation.

Consecutive karyotype diagnoses of trisomy 21 made after genetic amniocenteses at the Diagnostic Ultrasound Associates were identified for the period January 1, 1985, through October 1, 1986. Exclusion criteria were similar. Data were available from 20 patients with Down syndrome fetuses from 15 to 19 weeks' gestation.

Ultrasound techniques and equipment. The biparietal diameter was measured with electronic calipers from leading edge to leading edge at the level of the thalami. The femur was preferentially visualized from the greater trochanter to the end of the ossified shaft, perpendicular to the long axis of the bone, and its length was recorded with electronic calipers. The cephalic index was calculated by dividing the biparietal diameter by the occipitofrontal diameter measured at the same level.

The ultrasound equipment used in New Haven included the General Electric models RT 2600 and 3000 (3.5 and 5.0 mHz linear array transducers; Rancho Cardova, California), the Advanced Technology Laboratories Ultramark 4 (3.5 and 5.0 mHz linear array transducers; Botnell, Washington), and the Acuson 128 (3.0 mHz linear array transducer). The equipment used in Boston included an Acuson 128 or ATL 300 (3.0 and 3.5 mHz sector transducers). To ensure an accurate estimate of the femur length measurements with the sector scanner, the femur lengths were repeatedly imaged and the longest possible length was recorded.

Data collection and statistical analysis. Ultrasound

Table II. Comparisons between cases and controls at 16 and 17 weeks' gestation for the Boston population

Variable	*Gestational age (wk)*	*Group*	*No.*	*Mean*	*SD*	*t test*	*Wilcoxon test*
Gestational age	15.0-16.5	DS	8	15.7	0.47		
		NL	71	15.7	0.34	$p < 0.73$	$p < 0.95$
	16.6-17.5	DS	8	16.9	0.42		
		NL	80	16.8	0.37	$p < 0.50$	$p < 0.52$
Biparietal diameter	15.0-16.5	DS	8	3.31	0.12		
		NL	71	3.31	0.12	$p < 0.98$	$p < 0.86$
	16.6-17.5	DS	8	3.65	0.12		
		NL	80	3.62	0.13	$p < 0.57$	$p < 0.62$
Femur length	15.0-16.5	DS	8	1.85	0.19		
		NL	71	2.10	0.15	$p < 0.0001$	$p < 0.001$
	16.6-17.5	DS	8	2.15	0.24		
		NL	80	2.38	0.14	$p < 0.0001$	$p < 0.009$
Biparietal diameter/femur length ratio	15.0-16.5	DS	8	1.80	0.12		
		NL	71	1.59	0.10	$p < 0.0001$	$p < 0.0002$
	16.6-17.5	DS	8	1.71	0.17		
		NL	80	1.52	0.08	$p < 0.0001$	$p < 0.002$

DS = Down syndrome; NL = normal.

reports were reviewed and the indications for evaluation, gestational age, biparietal diameter, femur length, and cephalic index were recorded. The calculation of the cephalic index was not available for the Boston population. Hadlock's formula[6] and Jeanty's formula[7] were used in the estimation of gestational age based on biparietal diameter and femur length, respectively.

The biparietal diameter, femur length, biparietal diameter/femur length ratio, and cephalic index were compared in the Down syndrome and control populations at given gestational ages. Differences between cases and controls were identified by Student's *t* test and a Wilcoxon signed rank test. The ability of specific biparietal diameter/femur length cutoff values to discriminate between Down syndrome and normal fetuses was assessed by χ^2 analysis and Fisher's exact test. The effect of gestational age on the biparietal diameter/femur length ratio was assessed by general linear model regression analysis.

Results

There was a consistent underestimation of the femur length for a given gestational age in the New Haven population, and a consistent overestimation in the Boston population when compared with Jeanty's nomogram.[7] The differences between the two centers were statistically significant ($p < 0.0001$) from 16 to 19 weeks' gestation, and precluded combining the two centers' data sets.

New Haven results. Power analysis[4] demonstrated that adequate numbers of cases and controls were available to allow for statistically meaningful (>80%) comparisons from 16 to 17, 17.5 to 18.5, and 20 to 21 weeks' gestation (Table I). Other gestational week intervals contained insufficient cases. No statistically significant differences were detected between cases and controls for mean gestational age, suggesting similar gestational age distributions within the chosen intervals. No statistically significant differences were noted for cephalic index or biparietal diameter. There was, however, a statistically significant shortening of femur lengths in the Down syndrome fetuses in each gestational week interval. Furthermore, the biparietal diameter/femur length ratio resulted in the highest statistically significant differences between the two populations. A Wilcoxon signed rank test confirmed the statistically significant differences for femur length and particularly for the biparietal diameter/femur length ratio (Table I).

Boston results. Identical findings were noted for the Boston center (Table II). Gestational age intervals were again chosen to maximize power (>80%) and included 15.0 to 16.0 and 16.5 to 17.5 weeks' gestation. No significant differences were noted for gestational age or biparietal diameter between the case and control groups. Statistically significant differences were noted for femur length and particularly the biparietal diameter/femur length ratio between the two groups.

Variation in biparietal diameter/femur length ratios across gestational age. The biparietal diameter/femur length ratio (BPD/DL) was noted to decrease across gestational age (GA) in both centers' control populations according to the following formulas:

$$BPD/FL = 3.222 - 0.125\ (GA) + 0.002\ (GA)^2$$
$$BPD/FL = 2.406 - 0.052\ (GA)$$
$$(R^2 = 0.56;\ p < 0.0001;\ \text{New Haven})$$
$$(R^2 = 0.36;\ p < 0.0001;\ \text{Boston})$$

The biparietal diameter/femur length ratio was consistently higher in the Down syndrome population at

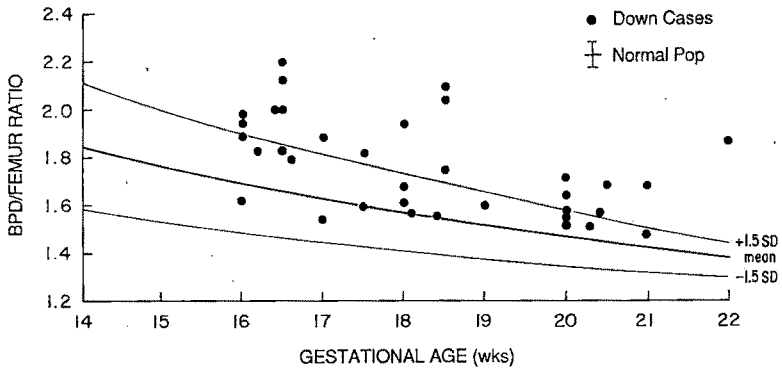


Fig. 1. The biparietal diameter/femur length ratio *(BPD/FEMUR)* in the normal population (±1.5 SD) and in Down syndrome cases for the New Haven population.

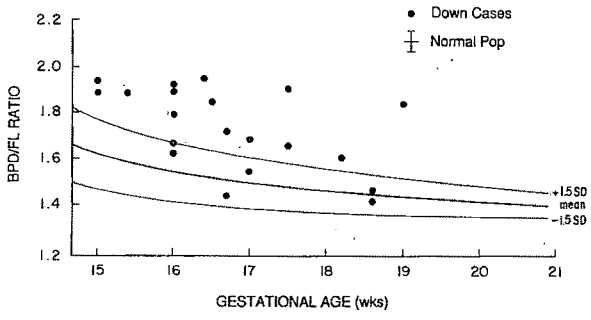


Fig. 2. The biparietal diameter/femur length ratio *(BPD/FL)* in the normal population (±1.5 SD) and in Down syndrome cases for the Boston population.

all gestational ages. For the New Haven population, 51% of Down syndrome cases were found to have a biparietal diameter/femur length ratio 1.5 SD above the control population mean for a given gestational age, while only 6% of Down syndrome fetuses were below the mean (Fig. 1). For the Boston population, 70% of Down syndrome fetuses were 1.5 SD above the control population mean, while only 10% were at or below the mean (Fig. 2).

Predictive value of biparietal diameter/femur length ratio in discriminating between Down syndrome and normal fetuses. The sensitivity and false positive rate of the biparietal diameter/femur length ratio in differentiating Down syndrome from normal fetuses were also evaluated for each center (Table III). With a cutoff value of 1.5 SD above the mean ratio for the normal New Haven population, 51% of Down syndrome fetuses were identified with a false positive rate of 7%. Given a general population incidence for Down syndrome at term of 1/1000[6] and a fetal loss rate of 29%,[5] the incidence of Down syndrome in the second trimester should be 1/710 and the resulting positive predictive value of the proposed biparietal diameter/femur length ratio cutoff would be 1/103. Thus 102 amniocenteses on non–Down syndrome gestations would be necessary for each Down syndrome diagnosis. In a high-incidence population, such as women 35 years old, the anticipated incidence of Down syndrome at the time of genetic amniocentesis would be 1/270,[8] and thus 37 amniocenteses would be required for one diagnosis of Down syndrome (positive predictive value 1/37).

For the Boston center, a cutoff value of 1.5 SD above the mean ratio for the control population would identify 70% of cases with a false-positive rate of 4.6%. The positive predictive value for the general obstetric population would be 1/47 and that for women at age 35 years would be 1/18.

The ability of the femur length measurement alone to differentiate Down syndrome from normal fetuses was also assessed. With a cutoff value of 1.5 SD below the control (New Haven) population mean, 40% of Down syndrome fetuses were identified, while for the Boston center 60% of Down syndrome fetuses were identified. Femur length, therefore, proved less accurate than the biparietal diameter/femur length ratio in discriminating Down syndrome fetuses.

Comment

Recently, characteristic sonographic "signs" for the Down syndrome fetus have been proposed as adjuncts to screening programs. The sonographic finding of posterior occipital-nuchal skin thickening may be present in up to 45% of Down syndrome fetuses in the second trimester.[9] The practical application of this observation remains to be determined. In addition, duodenal obstruction occurs in 8% of Down syndrome infants[10] and has been proposed as a marker for the condition.[11] Unfortunately, the literature suggests that the diagnosis of duodenal obstruction can rarely be made before 24 weeks' gestation.[12] Ventricular septal and atrioventricular canal defects are present in up to 50% of Down syndrome infants,[13] but it is not clear

Table III. Sensitivity and false-positive rate of the biparietal diameter/femur length ratio in the detection of Down syndrome

Test center	*Gestational age (wk)*	*No.*	*Cutoff value (mean + 1.5 SD)*	*Overall sensitivity*	*Overall false positive rate*	*p value**
New Haven	15.0-15.9	27	1.93			
	16.0-16.9	141	1.93			
	17.0-17.9	64	1.76			
	18.0-18.9	53	1.74	18/35	26/349	<0.0001
	19.0-19.9	27	1.68	(51%)	(7%)	<0.0001
	20.0-20.9	25	1.58			
	21.0-21.9	8	1.54			
	22.0-22.9	4	1.47			
Boston	15.0-15.9	36	1.77			
	16.0-16.9	78	1.66			
	17.0-17.9	50	1.61	14/20	9/195	<0.0001
	18.0-18.9	16	1.54	(70%)	(5%)	<0.0001
	19.0-19.9	11	1.51			
	20.0-20.9	4	1.48			

*χ^2 or Fisher's exact test.

what percentage of these defects can be identified with an ultrasound screening examination. The use of standard ultrasound biometry to differentiate Down syndrome from normal fetuses has great appeal. Both biparietal diameter and femur length are readily and accurately measured during a basic ultrasound examination. These measurements require neither sophisticated training nor expensive ultrasound equipment.

Initial efforts to define a biometric marker of Down syndrome used the long-standing observation that Down syndrome infants tend toward brachycephaly (increased cephalic index).[14] Perry et al.,[15] however, were not able to differentiate normal from Down syndrome fetuses on the basis of an increased cephalic index during second-trimester sonography. Our data confirm their observation, with no difference in cephalic index noted between case and control populations (Table I). In addition, we have observed that while femur lengths were significantly shorter in Down syndrome fetuses, consistent with their shorter stature,[16] femur length per se was not the optimal discriminator. By combining biparietal diameter and femur length measurements into a ratio, discrimination between Down syndrome and normal fetuses is enhanced. This finding is in agreement with the observations of Collett et al.,[17] who noted that the biparietal diameter/femur length ratio was elevated in fetal Down syndrome. This suggests that in the normal population, shorter femur lengths are associated with smaller biparietal diameters, perhaps as a consequence of inaccurate dating or symmetric growth retardation. Because the biparietal diameter/femur length ratio is far less affected by changes in gestational age than is femur length alone, it should provide a more reliable clinical marker for Down syndrome.

The use of the biparietal diameter/femur length ratio with a 1.5 SD cutoff, between 15 and 23 weeks' gestation, allows for the detection of Down syndrome in the general obstetric population with a sensitivity of 50% to 70%, greater than that of either low maternal serum α-fetoprotein concentration (≤0.5 multiple of the median) or maternal age–based (>34 years) screening programs alone (<20% each). In addition, the positive predictive value exceeds that of maternal age–based screening; thus a far larger number of Down syndrome fetuses are identified with fewer unnecessary amniocenteses.

A number of potential limitations are present in this study. The Down syndrome sample size is small. However, a power analysis suggests >90% probability of identifying a true difference between the Down syndrome and control populations.[18] An additional source of error may result from the tendency for sonographers to minimize gestational age discrepancies between biparietal diameter and femur length measurements. This phenomenon could cause a significant reduction in the ratio's sensitivity for the detection of Down syndrome, because the greater the biparietal diameter/femur length discrepancy, the greater the likelihood of remeasurement.

The difference in femur length measurements between the two centers probably reflects differences in methodology and not variations in population. The use of a sector transducer results in a relative overestimation of long bone length, in a plane perpendicular to the bone's long axis, when compared with the linear array transducer.[19] Furthermore, while the greater trochanter provides a ready landmark for measurement, sonographers may vary in their assessment of the distal termination of the femoral diaphysis. We postulate that an overestimation of femur length accounts for the Boston center's greater diagnostic sensitivity. The smaller biparietal diameter/femur length ratio in the Boston control population may paradoxically amplify

the effect of femur length shortening in the Down syndrome population. It is therefore crucial that each center establish its own biparietal diameter/femur length limits of confidence in the second trimester for Down syndrome risk assessment.

The role of the biparietal diameter/femur length ratio in prenatal screening for Down syndrome remains to be confirmed by further investigations. Variation in ultrasound equipment and technique may lead to significant effects on a given center's diagnostic efficacy. Nonetheless, this method appears at least comparable to methods that use maternal serum α-fetoprotein and/or maternal age. Since Baumgarten et al.[20] have demonstrated that ascertainment of Down syndrome risk by a combination of maternal serum α-fetoprotein level and maternal age may improve diagnostic accuracy, the addition of a third independent predictor (biparietal diameter/femur length ratio) of Down syndrome risk could further enhance this diagnostic accuracy. The effect of the biparietal diameter/femur length ratio on the relative risk of Down syndrome could be assessed and used to provide a patient with an adjusted risk based on all three parameters.

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Colposcopic correlates of cervical papillomavirus infection

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We compared the colposcopic and histologic features of 39 papillomavirus-related cervical lesions with their associated human papillomavirus type. Two thirds of the lesions contained human papillomavirus type 16–related deoxyribonucleic acid, and 69% of them were classified as cervical intraepithelial neoplasia I or II, in contrast to 92% of lesions containing human papillomavirus 6/11 deoxyribonucleic acid. On histologic examination, low-grade cervical intraepithelial neoplastic lesions tended to be sharply demarcated and have an uneven or granular surface contour. In contrast, high-grade lesions often had diffuse borders and were usually smooth in appearance. Both low- and high-grade lesions displayed variable degrees of whiteness, thickness, and papillomatous configurations. Patterns of coarse punctation were more common in higher grade cervical intraepithelial neoplastic lesions and in those containing human papillomavirus 16. Predictably, the colposcopic features of lesions containing human papillomavirus 6/11 deoxyribonucleic acid sequences compared closely with those of low-grade cervical intraepithelial neoplasia. In contrast, human papillomavirus 16–associated cervical intraepithelial neoplasia exhibited a greater spectrum of colposcopic patterns, including those associated with condyloma and low- and high-grade cervical intraepithelial neoplastic lesions. We conclude that, although a portion of low- and high-grade cervical intraepithelial neoplastic lesions can be distinguished colposcopically, a significant number of lesions containing features of low-grade cervical intraepithelial neoplasia or condyloma contain human papillomavirus 16 deoxyribonucleic acid and cannot be distinguished from presumably innocuous human papillomavirus infections. (Am J Obstet Gynecol 1987;157:809-14.)

Key words: Colposcopy, papillomaviruses, cervical intraepithelial neoplasia

In the past 10 years colposcopy has become an accepted and to most gynecologists necessary screening tool for evaluating women with abnormal Papanicolaou smears. With colposcopy it is possible to visualize directly precancerous lesions of the cervix and lower genital tract, to determine their distribution and extent, and to select the most appropriate areas for biopsy to ensure an accurate diagnosis. With this approach, it is possible to rule out cancer in a large proportion of cases, facilitating conservative ablation with cryotherapy or laser in most.[1-3]

The possibility that colposcopy may predict histologic changes has been suggested by some authors, who have equated increases in vascular atypia with increasing grade of cervical intraepithelial neoplasia or dysplasia.[1] With the discovery that papillomaviruses are associated with precancerous lesions of the cervix, attention has focused on distinguishing infections associated with presumably benign and potentially precancerous lesions. Reid et al.[3] introduced a comprehensive battery of colposcopic indices that they claimed would differentiate a large proportion of condylomatous or low-grade cervical intraepithelial neoplastic lesions from high-grade lesions or carcinoma in situ.[3] However, Barasso et al.[4] were unable to formulate reliable colposcopic criteria for this distinction.

The observation that certain papillomaviral deoxyribonucleic acid (DNA) strains are preferentially associated with cancer has prompted efforts to distinguish precancerous lesions associated with these strains. Human papillomavirus types 16, 18, 31, 35, and others have been associated with cancers and a spectrum of cervical intraepithelial neoplastic lesions, as opposed to human papillomaviruses 6 and 11 (and others), which are associated with condylomas.[5-12] However, low-grade cervical intraepithelial neoplastic lesions and condylomas contain many common features and may be difficult to distinguish on colposcopic examination. Accordingly, it is uncertain whether colposcopy is sufficiently sensitive to distinguish cervical lesions associated with different human papillomavirus types.[3, 4, 11, 12]

Our purpose was to determine if a battery of colposcopic indices would distinguish between infections

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**Recipient of a Physician Scientist Award from the National Institutes of Health.*



Fig. 1. This cervical intraepithelial neoplasia 1/condyloma lesion is sharply demarcated and contains two growth patterns, including mosaiform *(lower right)* and papillomatous areas *(center and bottom left)*. DNA from this lesion contained sequences closely related to human papillomavirus 6/11 DNA.



Fig. 3. This cervical intraepithelial neoplasia 1/condyloma lesion is poorly demarcated anteriorly, with snow-white epithelium from the 6 to 9 o'clock positions. The human papillomavirus DNA from this lesion was closely related to human papillomavirus 6/11.



Fig. 2. This cervical intraepithelial neoplasia 2 lesion is sharply demarcated, with a prominent mosaiform pattern similar to that in Fig. 1. DNA from this lesion contained sequences closely related to human papillomavirus 16.

associated with human papillomavirus type 16 and those associated with types 6 and 11, the latter commonly found in condylomas of the lower genital tract. We present evidence that infections associated with different human papillomavirus types exhibit a wide array of colposcopic abnormalities and that their distinction is not always possible. Our inability to separate these processes may relate to a number of factors, including the morphologic similarities between all papillomavirus infections.

Material and methods

Selection of case material. All patients selected were private patients referred to one of the coauthors (R. U. L.) for the evaluation of an abnormal Papanicolaou smear. All patients underwent colposcopy, colpophotography, and colposcopically directed biopsies. Patients were included in this study if the colposcopy, photographic slide, and biopsy results were satisfactory and if analysis of their biopsy samples disclosed the presence of a papillomaviral DNA. The DNA hybridizations were performed in close collaboration with Dr. Saul Silverstein at the Cancer Research Center, Columbia University College of Physicians and Surgeons.

Colposcopic classification. Colposcopic classification of the cervical lesions was based on review of the slides



Fig. 4. This cervical intraepithelial neoplasia 1/condyloma lesion contains multiple areas of well demarcated thick white epithelium with prominent coarse punctation. The human papillomavirus DNA from this lesion was of undetermined type.



Fig. 5. This cervical intraepithelial neoplasia 3 lesion is poorly demarcated, with thin, gray-white epithelium from the 1 to 3 o'clock positions. The human papillomavirus DNA isolated from this lesion was closely related to human papillomavirus 16.

only. Three of us (M. F., R. U. L., and C. P. C.) reviewed the slides and classified the abnormalities according to a modification of the indices previously proposed.[3] These included degree of whiteness, demarcation, thickness, certain features of surface contour, and vascular changes. Vascular changes were characterized as absent, mosaiform or horizontal, fine punctation or vertical (including warty), true mosaic, coarse punctation, and atypical vessels (Figs. 1 to 6). Coarse punctation consisted of larger caliber vessels with irregular intercapillary distances. The colposcopic findings were then correlated with both the histologic diagnosis and the human papillomavirus DNA type detected in the tissue.

Histologic classification. All biopsy specimens were reviewed by two of us (C. P. C. and R. M. R.) and classified as negative (i.e., epidermidization, squamous metaplasia, or parakeratosis), or cervical intraepithelial neoplastic lesion type 1, 2, or 3. For the purposes of this study, the distinction between low-grade cervical intraepithelial neoplasia and flat condyloma was not made, becuase they share similar morphologic features and may at times be difficult to distinguish from one another.[12]

Analysis for human papillomavirus DNA. One half of each biopsy sample was frozen, examined by frozen section, then analyzed by Southern blot hybridization for the presence of human papillomavirus DNA sequences as described previously.[10] With a series of low stringent hybridizations with types 6 and 11, 16, and 18, tissue DNA samples were screened for the presence of human papillomavirus DNA sequences followed by exposure of hybridized filters to x-ray film. After exposure, the filters were washed under stringent conditions, reexposed, and the hybridization signals were compared. If signals remained strong under both conditions, the human papillomavirus DNA was designated as closely related to the probe used. If the signal significantly diminished after the stringent wash, the virus was assumed to be different from the probe(s) used. The human papillomavirus DNA samples were thus designated as one of the following: type 6 or 11, type 16, type 18, a mixture of more than one type, and a type other than 6, 11, 16, and 18.

Results

Patient population. Forty-four patients were included in the study. They ranged in age from 20 to 58 years (mean 33 years). All were referred because of an abnormal Papanicolaou smear. After biopsy and treatment, the patients were followed up to 36 months (mean 10 months).

Histopathology. Smears were taken at the time of



Fig. 6. This lesion contains a "true mosaic" vascular pattern, characterized by thick white epithelium and an interlacing network of prominent vessels accompanied by punctation. This was a cervical intraepithelial neoplasia type 3 lesion with gland involvement. Viral studies were not performed in this case.

colposcopy and biopsy from 34 patients. They included three normal, 13 with atypia suggesting grade 1 cervical intraepithelial neoplasia or condyloma, 18 with grade 1 cervical intraepithelial neoplasia, five with grade 2 cervical intraepithelial neoplasia, and five with grade 3 cervical intraepithelial neoplasia. Biopsy information was available from all patients. Twenty-two were diagnosed as having grade 1 cervical intraepithelial neoplasia or condyloma, 13 as having grade 2 lesions, and nine as having grade 3 lesions (Table I).

DNA hybridization. The viral DNA types detected are shown in Table I. Thirteen lesions contained sequences closely related to human papillomaviruses 6 or 11, 26 were closely related to type 16, two lesions contained a mixture of types, including 6/11 and 16, and two lesions contained types not closely related to the probes used.

Correlation of lesion grade with colposcopic index. Most of the lesions, including all grades, were gray-white in color (Table II). Most grade 1 cervical intraepithelial neoplasia lesions were sharply demarcated, whereas most grade 3 lesions had diffuse borders. Equal proportions of lesions were thick and thin, except for grade 3 lesions, most of which were thin. Most

Table I. Diagnosis versus human papillomavirus DNA type

	Cervical intraepithelial neuroplasia			
Viral DNA type	*Grade 1**	*Grade 2*	*Grade 3*	*Total*
16	8	10	8	26
6/11	10	2	1	13
16/11	1	1	—	2
Not determined†	3	—	—	3
	22	13	9	44

*For these purposes flat condyloma and grade cervical intraepithelial neuroplasia with koilocytotic atypia were classified under grade 1 cervical intraepithelial neuroplasia.

†Human papillomavirus type other than types 6, 11, or 16.

grade 1 lesions had an uneven or granular surface contour, in contrast to type 3 lesions, which tended to be smooth. Both grades 1 and 2 lesions contained papillomatous features. Certain vascular changes, including primarily mosaiform or no abnormal vascular pattern, were evenly distributed among all three grades. In contrast, coarse punctation was seen in 19% of higher grade cervical intraepithelial neoplasia versus 6% of low-grade cervical intraepithelial neoplasia/condyloma.

Correlation of viral DNA strain with colposcopic index. Most of the lesions containing human papillomavirus 16 DNA sequences were gray-white, while lesions containing type 6/11 sequences were nearly equally divided between gray-white and snow white (Table II). Although human papillomavirus 16–associated lesions were nearly equally divided between sharp and diffuse, most 6/11–associated lesions were sharply defined. The majority of all lesions were thin, with a rough or uneven surface contour. Interestingly, the majority of type 16–associated lesions did not display marked vascular atypia, because the majority of all lesions contained either a mosaiform vascular pattern or no significant vascular changes. Predictably, a greater percentage of lesions containing type 16 (16%) contained coarse punctation, in contrast to those containing type 6/11 (8%). A single human papillomavirus type 16–containing lesion with true vascular atypia was a grade 3 cervical intraepithelial neoplasia lesion that also contained invasive carcinoma.

Comment

Our goal was to determine if cervical lesions associated with specific human papillomavirus DNA strains could be predicted from colposcopic examinations. There is evidence that both the grade of a lesion and its associated viral type may influence the risk of progression to cancer if the lesion is not treated. Colposcopy, as designed, is part of a diagnostic protocol and, irrespective of the results of this or any other study, we would not recommend that it be used alone to decide

Table II. Colposcopy versus lesion and DNA type (expressed as percent)

	Cervical intraepithelial neoplasia			*Human papillomavirus*	
Colposcopic parameter	*Grade 1 (n = 18)*	*Grade 2 (n = 12)*	*Grade 3 (n = 9)*	*Type 16 (n = 26)*	*Type 6/11 (n = 13)*
Color					
Snow white	31	23	22	19	46
Gray-white	50	69	55	61	38
Intermediate	18	8	22	15	15
Demarcation					
Sharp	68	53	33	46	61
Diffuse	31	46	66	53	38
Thickness					
Thick	50	46	33	38	38
Thin	50	53	66	61	61
Surface contour					
Uneven or granular	68	0	22	38	83
Papillomatous	9	23	0	19	7
Vascular changes					
Absent	33	33	33	37	23
Fine punctation	17	33	11	12	23
Coarse punctation	6	16	22	16	8
Mosaiform	44	33	33	37	46
Mosaic	6	0	11	4	7
Atypical vessels	0	0	11	4	0

if a cervical lesion was high or low risk, or if it should be removed. Nevertheless, in view of the close association between histology, viral DNA type, and biologic behavior, understanding the relationship of colposcopic presentation to histologic and viral parameters is important.[5-12]

As shown by the correlations in Table II, we were not able to establish a strong relationship between the colposcopic parameters outlined and lesion grade. This lack of a precise correlation parallels a recent study by Barasso et al.,[4] who found that many cervical intraepithelial neoplastic lesions shared the colposcopic features of condyloma. They found that three parameters associated with condyloma—irregular staining with Lugol's stain, the presence of asperites, and satellite lesions—were found in over two thirds of lesions with the histologic features of cervical intraepithelial neoplasia. This is in contrast to Reid et al.[3] who found that colposcopic indices could usually distinguish between lesions at the low (condyloma and grade 1 cervical intraepithelial neoplasia) and high (grade 3 cervical intraepithelial neoplasia) end of the spectrum. Although we were not able to confirm the latter findings, it is important to stress that our study consisted of lesions presenting primarily with low-grade Papanicolaou smears (grade 1 cervical intraepithelial neoplasia or condyloma) and lesions on the lower end of the cervical intraepithelial neoplasia spectrum. Moreover, most did not display prominent vascular changes on colposcopic examination. The vascular changes seen in this population consisted primarily of horizontal capillary arcades (mosaiform), which we and others have distinguished from the more classical mosaic traditionally associated with higher grade precancerous lesions[1, 3] (Figs. 1, 2, and 6). As would be expected, coarse punctation was more commonly observed in higher-grade lesions and in those containing human papillomavirus 16. However, most lesions were in the lower end of the cervical intraepithelial neoplasia spectrum, and this feature was not prevalent enough to serve as a good discriminator of lesion grade. Thus, because there were few high-grade lesions in our study for comparison, it would be premature to state that most low-grade lesions cannot be distinguished from high-grade cervical intraepithelial neoplasia.

The important finding in our study is the presence of human papillomavirus type 16 DNA in both low- and high-grade cervical intraepithelial neoplasia and in lesions displaying a wide spectrum of colposcopic features. Human papillomavirus 16 has been associated with high-grade precancerous lesions (cervical intraepithelial neoplasia grade 3) and invasive cancers.[6] We and others have found that human papillomavirus 16 DNA is the human papillomavirus DNA that is most commonly isolated from cervical lesions, including many lesions that exhibit features of both condyloma and cervical intraepithelial neoplasia.[9-12] This would explain the association of type 16 with many lesions classified as grade 1 cervical intraepithelial neoplasia in our study, as well as the close correlation between type 16 and lesions with colposcopic features associated with low-grade cervical intraepithelial neoplasia or condyloma.

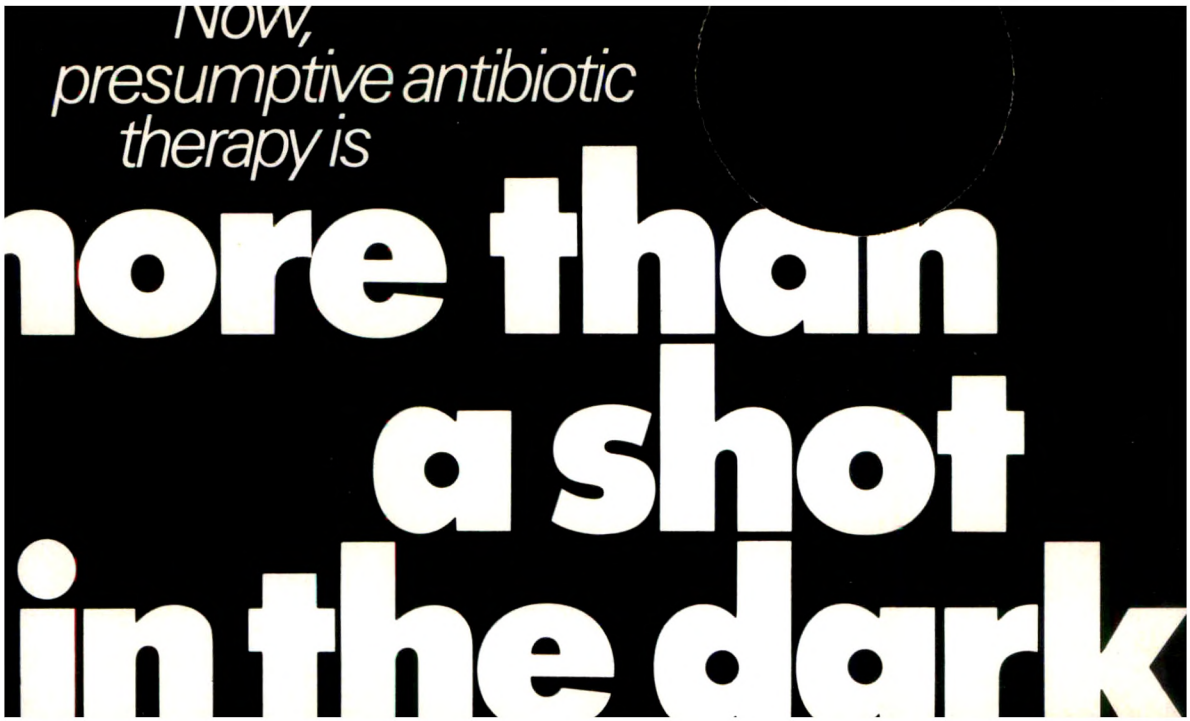
The fact that human papillomavirus 16 DNA is found in a wider spectrum of cervical intraepithelial neoplastic lesions than type 6/11 may explain some of the col-

poscopic findings. For example, most low-grade cervical intraepithelial neoplastic lesions (including condylomas) were sharply demarcated, whereas high-grade lesions tended to have diffuse borders. Accordingly, lesions containing type 6/11 were usually sharply demarcated, in keeping with the association of type 6/11 with low-grade cervical intraepithelial neoplasia or condyloma. However, type 16–related lesions were equally sharp and diffuse in appearance, reflecting the association of type 16 with a broader spectrum of colposcopic abnormalities, including both low- and high-grade cervical intraepithelial neoplasia. As mentioned, coarse punctation, while more common in human papillomavirus type 16—containing lesions, was infrequently present in all lesions, many of which contained type 16.

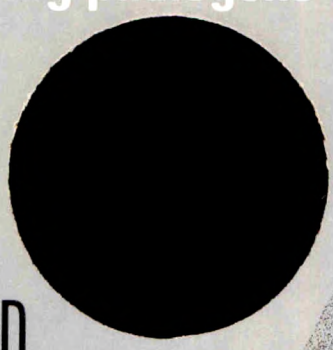
The morphologic overlap between low-grade cervical intraepithelial neoplasia and condyloma and the association of human papillomavirus 16 DNA with lesions throughout the cervical intraepithelial neoplasia spectrum emphasizes the difficulty of distinguishing all potential cancer precursors at the colposcopic level. This problem of recognition is further compounded by the possibility of clinically and histologically latent human papillomaviral infection, the natural history of which is still unknown.[13, 14] This would place the value of colposcopy primarily at detecting lesions and distinguishing their limits and any areas within the lesion containing abnormal vessels that might represent invasive cancer. Given that potentially oncogenic human papillomavirus DNA strains are found in all grades of precancers (including normal-appearing mucosa), colposcopy is not an appropriate method by itself for predicting the natural history of a cervical lesion.

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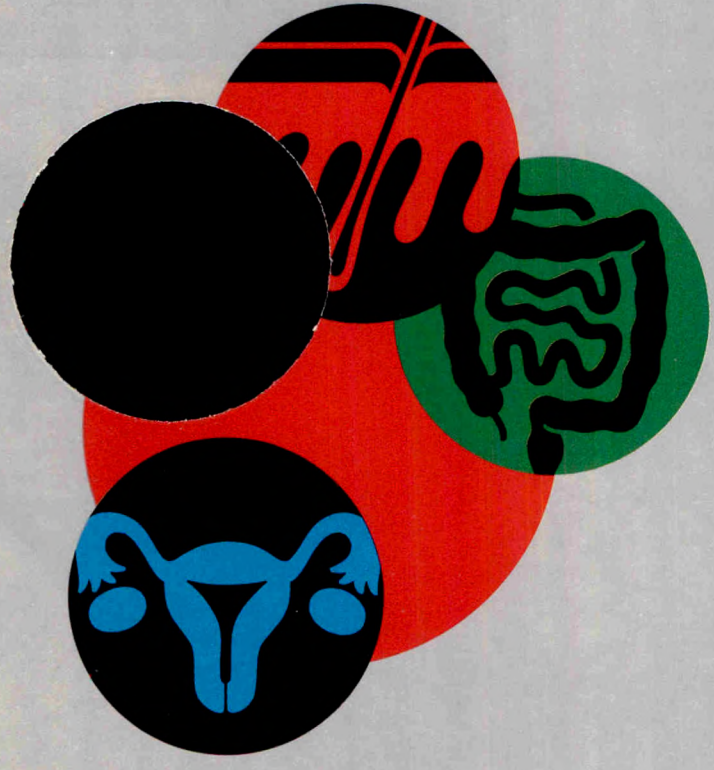
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Bacteriologic efficacy	**93.3%** (528/566 isolates)	eradicated	**90.2%** (248/275 isolates)
Clinical efficacy	**98.3%** (231/235 patients)	satisfactory response	**97.1%** (136/140 patients)
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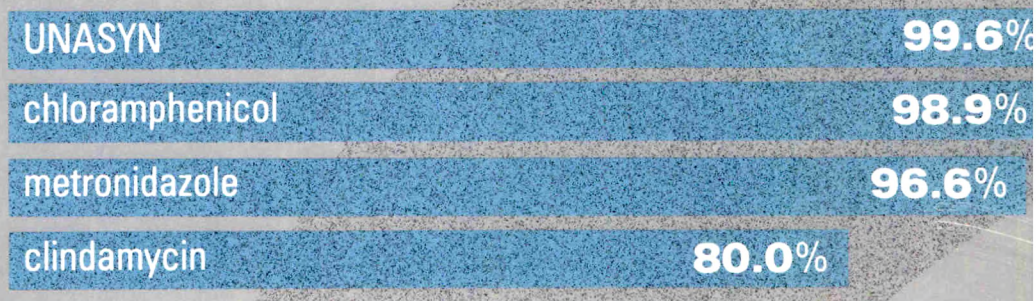
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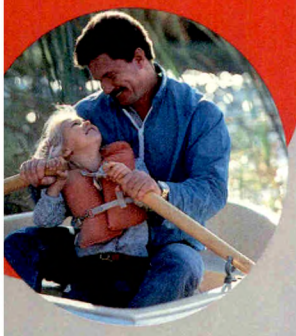
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ANTIBIOTIC POWER

References:

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BRIEF SUMMARY
UNASYN® (ampicillin sodium/sulbactam sodium) IM/IV, 1.5 and 3g vials

Indications and Usage
UNASYN is indicated for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions listed below.

Skin and Skin Structure Infections caused by beta-lactamase producing strains of *Staphylococcus aureus*, *Escherichia coli*,* *Klebsiella* spp.* (including *K. pneumoniae**), *Proteus mirabilis*,* *Bacteroides fragilis*,* *Enterobacter* spp.,* and *Acinetobacter calcoaceticus*.*

Intra-Abdominal Infections caused by beta-lactamase producing strains of *Escherichia coli*, *Klebsiella* spp. (including *K. pneumoniae**), *Bacteroides* spp. (including *B. fragilis*), and *Enterobacter* spp.*

Gynecological Infections caused by beta-lactamase producing strains of *Escherichia coli*,* and *Bacteroides* spp.* (including *B. fragilis**).

*Efficacy for this organism in this organ system was studied in fewer than 10 infections.

While UNASYN is indicated only for the conditions listed above, infections caused by ampicillin-susceptible organisms are also amenable to treatment with UNASYN due to its ampicillin content. Therefore, mixed infections caused by ampicillin-susceptible organisms and beta-lactamase producing organisms susceptible to UNASYN should not require the addition of another antibiotic.

Contraindications
The use of UNASYN is contraindicated in individuals with a history of hypersensitivity reactions to any of the penicillins.

Warnings
SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (ANAPHYLACTIC) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE APT TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR HYPERSENSITIVITY REACTIONS TO MULTIPLE ALLERGENS. THERE HAVE BEEN REPORTS OF INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY WHO HAVE EXPERIENCED SEVERE REACTIONS WHEN TREATED WITH CEPHALOSPORINS. BEFORE THERAPY WITH A PENICILLIN, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS, AND OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, UNASYN SHOULD BE DISCONTINUED AND THE APPROPRIATE THERAPY INSTITUTED.

SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN, INTRAVENOUS STEROIDS, AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.

Precautions
General: A high percentage of patients with mononucleosis who receive ampicillin develop a skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. In patients treated with UNASYN, the possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.

Drug Interactions: Probenecid decreases the renal tubular secretion of ampicillin and sulbactam. Concurrent use of probenecid with UNASYN may result in increased and prolonged blood levels of ampicillin and sulbactam. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with UNASYN and allopurinol administered concurrently. UNASYN and aminoglycosides should not be reconstituted together due to the *in vitro* inactivation of aminoglycosides by the ampicillin component of UNASYN.

Drug/Laboratory Test Interactions: Administration of UNASYN* (ampicillin sodium/sulbactam sodium) will in high urine concentration of ampicillin. High urine concentrations of ampicillin may result in false p reactions when testing for the presence of glucose in urine using Clinitest™, Benedict's Solution or Fe Solution. It is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clini or Testape™) be used. Following administration of ampicillin to pregnant women, a transient decrease in p concentration of total conjugated estriol, estriolglucuronide, conjugated estrone, and estradiol has been This effect may also occur with UNASYN.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been perfor evaluate carcinogenic or mutagenic potential.

Pregnancy
Pregnancy Category B: Reproduction studies have been performed in mice, rats, and rabbits at doses up (10) times the human dose and have revealed no evidence of impaired fertility or harm to the fetus UNASYN. There are, however, no adequate and well controlled studies in pregnant women. Because a reproduction studies are not always predictive of human response, this drug should be used during pregnan if clearly needed. (See—Drug/Laboratory Test Interactions.)

Labor and Delivery: Studies in guinea pigs have shown that intravenous administration of ampicillin decreas uterine tone, frequency of contractions, height of contractions, and duration of contractions. However, it known whether the use of UNASYN in humans during labor or delivery has immediate or delayed adverse effe the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obst intervention or resuscitation of the newborn will be necessary.

Nursing Mothers: Low concentrations of ampicillin and sulbactam are excreted in the milk; therefore, c should be exercised when UNASYN is administered to a nursing woman.

Pediatric Use: The efficacy and safety of UNASYN have not been established in infants and children under th of 12.

Adverse Reactions
UNASYN is generally well tolerated. The following adverse reactions have been reported:

Local Adverse Reactions
Pain at IM injection site—16%
Pain at IV injection site—3%
Thrombophlebitis—3%

Systemic Adverse Reactions
The most frequently reported adverse reactions were diarrhea in 3% of the patients and rash in less than the patients.

Additional systemic reactions reported in less than 1% of the patients were: itching, nausea, vomiting didiasis, fatigue, malaise, headache, chest pain, flatulence, abdominal distension, glossitis, urine rete dysuria, edema, facial swelling, erythema, chills, tightness in throat, substernal pain, epistaxis, and m bleeding.

Adverse Laboratory Changes
Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were

Hepatic: Increased AST (SGOT), ALT (SGPT), alkaline phosphatase, and LDH.

Hematologic: Decreased hemoglobin, hematocrit, RBC, WBC, neutrophils, lymphocytes, platelets and incr lymphocytes, monocytes, basophils, eosinophils, and platelets.

Blood Chemistry: Decreased serum albumin and total proteins.

Renal: Increased BUN and creatinine.

Urinalysis: Presence of RBC's and hyaline casts in urine.

The following adverse reactions have been reported with ampicillin-class antibiotics and can also occu UNASYN.

Gastrointestinal: Gastritis, stomatitis, black "hairy" tongue, enterocolitis and pseudomembranous colitis.

Hypersensitivity Reactions: Urticaria, erythema multiforme, and an occasional case of exfoliative dermatiti been reported. These reactions may be controlled with antihistamines and, if necessary, systemic corticoste Whenever such reactions occur, the drug should be discontinued, unless the opinion of the physician di otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with a penicilli WARNINGS).

Hematologic: In addition to the adverse laboratory changes listed above for UNASYN, agranulocytosis has reported during therapy with penicillins. All of these reactions are usually reversible on discontinuation of th and are believed to be hypersensitivity phenomena.

NEW Unasyn® IM IV

(ampicillin sodium/sulbactam sodium)

On target in mixed infection†

†Due to susceptible strains of indicated organisms.

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Infection and labor: The detection of endotoxin in amniotic fluid

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Intra-amniotic infections are frequently caused by gram-negative organisms. Lypopolysaccharide, or endotoxin, is a component of the cell wall of these bacteria. The *Limulus* amebocyte lysate assay is the most sensitive test clinically available for the detection of endotoxin in biologic samples. The diagnostic value of the *Limulus* amebocyte lysate assay test for the detection of intra-amniotic infection has been examined and compared with that of the Gram stain. The Gram stain had a 60.8% sensitivity and 9.6% false positive rate. The *Limulus* amebocyte lysate assay had a 69% sensitivity and a 4.8% false positive rate. When both tests were used in combination, the sensitivity improved to 95.6%, whereas the false positive rate was 14.3%. The *Limulus* amebocyte lysate assay test is a rapid, sensitive, inexpensive, and simple test for the detection of gram-negative intra-amniotic infections. (AM J OBSTET GYNECOL 1987;157:815-9.)

Acute bacterial intra-amniotic infections are an important cause of perinatal morbidity and death.[1] Although early diagnosis and aggressive treatment are believed to decrease the ill effects of intra-amniotic infection, this goal is frequently difficult to achieve because the infection may not be clinically apparent before delivery in all patients.[2] It is likely that bacterial colonization of the amniotic cavity may be detectable before the appearance of overt clinical signs of infection such as fever, uterine tenderness, maternal and fetal tachycardia, and maternal leukocytosis.[3,4]

Because of the potentially serious consequences associated with delayed diagnosis, several tests for the early diagnosis of intra-amniotic infection have been developed. These include Gram stain examination of amniotic fluid, amniotic fluid leukocyte count, bacterial culture, serum C-reactive protein level, and gas liquid chromatographic analysis of amniotic fluid. Unfortunately, leukocyte count and C-reactive protein levels are nonspecific indicators of infection, the Gram stain is a relatively insensitive test, bacterial culture results are not available immediately, and gas liquid chromatographic analysis requires a costly apparatus.

In those cases in which intra-amniotic infection is associated with a gram-negative organism, the presence of bacterial endotoxin or lipopolysaccharide in the amniotic fluid could be anticipated. A rapid bioassay for lipopolysaccharide has been developed based on the gelation of the lysate of blood cells (amebocytes) of the horseshoe crab, *Limulus polyphemus*.[6] The purpose of this communication is to examine the value of this test for detecting lipopolysaccharide in amniotic fluid as an indicator of intra-amniotic infections.

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Material and methods

Amniotic fluid was obtained by amniocentesis from patients with preterm labor, premature rupture of membranes, or clinical suspicion of chorioamnionitis because of fever of unknown origin.

Gram stain examination. Gram stain examination of unspun amniotic fluid was performed by using commercial reagents (crystal violet, saffranin, and Gram's iodine) (Difco Laboratories, Detroit, Michigan) under standard conditions.[7] Stained slides were examined by trained technologists. The presence or absence of microorganisms and white blood cells was noted.

***Limulus* amebocyte lysate assay.** The *Limulus* amebocyte lysate assay was performed by adding 100 μl amniotic fluid to 100 μl *Limulus* amebocyte lysate (Haemachem Inc., St. Louis, Missouri, or Associates of Cape Cod, Woodshole, Massachusetts) and incubating the mixture in a motionless water bath at 37° C for 1 hour. A positive test result was scored when a solid adherent gel was present on inversion of the tube. A positive control (100 pg endotoxin) and a negative control (pyrogen-free water) were run with each assay.

Because the *Limulus* amebocyte lysate assay is a clot

Table I. *Limulus* amebocyte lysate assay results according to amniotic fluid culture

	Positive LAL	*Negative LAL*
Culture		
Positive	16	7
Negative	4	38
Total	20	45

LAL, *Limulus* amebocyte lysate assay.

Sensitivity: 16/23 = 69%; specificity: 38/42 = 95.2%; positive predictive value = 80%; negative predictive value = 84%; percent agreement = 83%; κ index = 0.62 (SE = 0.01); p = <0.00003.

Table II. Gram stain (bacteria) and amniotic fluid culture results

	Positive gram stain	*Negative gram stain*
Culture		
Positive	14	9
Negative	4	38
Total	18	47

Sensitivity: 14/23 = 60.8%; specificity: 38/42 = 90.4%; positive predictive value = 14/18 = 77.7%; negative predictive value = 38/47 = 80. 8%; percent agreement = 80%; κ index = 0.54 (SE = 0.11); p = <0.00003.

assay and amniotic fluid has recognized thromboplastic properties, we examined samples of this fluid from 76 patients undergoing amniocentesis for lung maturity. Inducers of the gelation of the *Limulus* amebocyte lysate assay were not identified in these culture-negative amniotic fluids.

Criteria for the diagnosis of chorioamnionitis, puerperal endometritis, and neonatal sepsis. Intra-amniotic infection was diagnosed by the presence of a positive amniotic fluid culture. Clinical chorioamnionitis was defined following the criteria proposed by Gibbs et al.[8] The diagnosis required a temperature elevation to 37.8° C and two or more of the following criteria: uterine tenderness, malodorous vaginal discharge, and leukocytosis. Leukocytosis was defined as a white blood cell count >15,000/mm. Endometritis was defined as postpartum temperature elevation to 38° C or higher on two occasions 4 hours apart, excluding the day of delivery, with uterine tenderness, foul-smelling lochia, and no other apparent source of fever. Neonatal sepsis was diagnosed in the presence of a positive culture of blood, urine, or cerebrospinal fluid.

Statistical methods. Diagnostic indexes (sensitivity, specificity, and positive and negative predictive value) for the Gram stain and *Limulus* amebocyte lysate assay were calculated according to previously described formulas.[9] Because the different methods of testing for intra-amniotic fluid infection were carried in the same experimental units, statistical significance tests for correlated samples were used. These test the null hypothesis that the marginal totals of the contingency table are homogeneous (i.e., are samples of the same population). McNemar's test was used for 2 × 2 tables and Cochran's Q statistics (which is distributed asymptotically as a χ^2 with n^{-1} degrees of freedom) for K × 2 tables. The unweighted κ statistic was used as a measure of agreement between two tests performed in the same experimental units.[10] Unlike measures of association in a contingency table, this statistic is corrected for the chance agreement that can be expected to occur given the marginal totals. The Fischer's exact test was employed to explore the relationship between *Limulus* amebocyte lysate assay, culture, and Gram stain results and clinical chorioamnionitis and endometritis.

Results

Sixty-five transabdominal amniocenteses were performed in 65 patients. The indications for amniocentesis were as follows: premature labor (n = 3), premature rupture of membranes (n = 51), and fever (n = 1). Culture, *Limulus* amebocyte lysate assay, and Gram stain results were available for all 65 specimens but in four cases no record was made about the presence or absence of white blood cells.

Intra-amniotic infection, defined as a positive amniotic fluid culture, was present in 23 cases. A single organism was isolated from 15 amniotic fluid specimens as follows: *Hemophilus vaginalis* (four cases), *Bacteroides* species (one case), *Staphylococcus aureus* (two cases), group B streptococcus (one case), *Streptococcus pneumoniae* (one case), *Neisseria gonorrhoeae* (one case), *Peptococcus* species (one case), *Escherichia coli* (one case), *Candida* (one case), *H. influenzae* (one case), and *Mycoplasma* species (one case). Two different organisms were isolated from eight specimens. The following bacteria were isolated: fusobacterium species (two cases), *Gardnerella vaginalis* (four cases), *B. fragilis* (five cases), *S. viridans* (four cases), *Lactobacillus* (one case), *Peptococcus* species (one case), *S. aureus* (two cases), and enterococcus (two cases).

Tables I and II depict the classification of culture-positive and culture-negative specimens on the basis of the *Limulus* amebocyte lysate assay and the presence or absence of bacteria based on the Gram stain.

The presence of bacteria in the Gram stain had a sensitivity of 60.8% and a false positive rate of 9.6%. The *Limulus* amebocyte lysate assay had a sensitivity of 69.5% and a false positive rate of 4.8% (Tables I and II). The agreement between the *Limulus* amebocyte lysate assay and the culture result was 83% and between

Table III. Correlation between the results of Gram stain and LAL of amniotic fluid and culture

	Gram stain result	
	*Correct**	*Incorrect*
LAL		
Correct*	44	10
Incorrect	8	3

LAL, *Limulus* amebocyte lysate assay.

*Correct indicates that the test (LAL or Gram stain) correlated appropriately with the result of amniotic fluid culture.

Table IV. LAL and Gram stain

	Positive	*Negative*	*Total*
Culture			
Positive	22	1	23
Negative	6	36	42
Total	28	37	65

LAL, *Limulus* amebocyte lysate assay.

Sensitivity: 22/23 = 95.6%; specificity: 36/42 = 85.7%; positive predictive value = 22/28 = 71.4%; negative predictive value = 36/37 = 97.2%; κ index = 0.77 (SE = 0.08); p = <0.00003.

the Gram stain and the culture result 80%. Table III shows how the *Limulus* amebocyte lysate assay and the Gram stain agreed with the culture result for each of the 65 samples on which both tests were carried out. The difference between the two tests was not statistically significant (McNemar's test).

When the results of the two tests were combined so that a positive Gram stain was indicative of intra-amniotic infection, regardless of the *Limulus* amebocyte lysate assay result, and a positive *Limulus* amebocyte lysate test indicative of intra-amniotic infection, regardless of the Gram stain result (a decision that was made a priori), the sensitivity increased to 95% whereas the false positive rate was 14.3% (Table IV).

The *Limulus* amebocyte lysate test is expected to detect gram-negative organisms. Because endotoxin is present only in gram-negative bacteria, false negative results might occur with intra-amniotic infections caused by gram-positive bacteria, mycoplasmas, and fungi. Table V displays *Limulus* amebocyte lysate results according to taxonomic classification and staining properties. The *Limulus* amebocyte lysate assay detected 33.3% (2/6) pure gram-positive infections whereas it was positive in 80% (12/15) of infections including a gram-negative organism (mixed infections and those including only gram-negative organisms). Of interest is that both *Candida* and *Mycoplasma* infections yielded a positive *Limulus* amebocyte lysate assay.

Three patients had false positive *Limulus* amebocyte lysate test results. All of them had premature rupture of membranes and went into labor within 24 hours of amniocentesis. None had clinical evidence of chorioamnionitis or postpartum endometritis nor their infants neonatal sepsis. In one case a Gram stain was positive for bacteria but culture failed to grow any organisms.

Chorioamnionitis was diagnosed in 26% (17/65) of cases, endometritis in 14.2% (8/56) of cases (nine patients with preterm labor were discharged undelivered; 56 + 9 = 65), and neonatal sepsis in only one case. The infant had a cerebrospinal fluid culture positive for *S. pneumoniae*. In this case the amniotic fluid had a positive Gram stain, the *Limulus* amebocyte lysate assay was negative, and the culture of amniotic fluid was positive for *S. pneumoniae*.

Table VI describes the relationship between Gram stain, *Limulus* amebocyte lysate assay, culture, and the presence of clinical chorioamnionitis and postpartum endometritis. A positive Gram stain or culture was associated with a higher incidence of clinical chorioamnionitis (Gram stain, p = 0.0031; culture, p = 0.0067; Fischer's exact test), whereas the *Limulus* amebocyte lysate assay was not (p = 0.127). On the other hand, the result of *Limulus* amebocyte lysate assay and culture (but not of the Gram stain) correlated with the presence of postpartum endometritis (Gram stain, p = 0.20; *Limulus* amebocyte lysate, p = 0.019; culture, p = 0.0002; Fischer's exact test).

Comment

Accumulating evidence suggests a role for infection in the cause of premature rupture of membranes and preterm labor. Several authors have proposed the use of amniocentesis for bacteriologic examination of the amniotic fluid in these circumstances.[3-5] Because culture results are not available immediately, initial management decisions are based on the Gram stain examination. A limitation of this approach is that the Gram stain examination of amniotic fluid is an insensitive technique detecting only 60% of all positive intra-amniotic infections.[17] This is a serious drawback because infants born to a mother with a false negative Gram stain have a similar rate of neonatal sepsis as that of infants with a true positive Gram stain.[17] Therefore, more sensitive methods for the rapid detection of bacteria in amniotic fluid are required.

Gram-negative organisms are frequently found in intra-amniotic infections. Their cell walls contain lipopolysaccharide, which can be detected as part of the intact cell wall or free after bacterial lysis. The *Limulus* amebocyte lysate assay test is the most sensitive technique currently available for detection of lipopolysaccharide in clinical specimens.[11] It can detect quantities of lipopolysaccharide as low as 5 pg/ml in water and 50 pg/ml in amniotic fluid.[12]

Our data show that the Gram stain and the *Limulus*

Table V. Gram stain and *Limulus* amebocyte lysate assay results according to taxonomic classification of organism and culture result

Taxonomic/staining classification of the organism	*Gram stain*			*LAL*		
	Positive	*Negative*	*Sensitivity (%)*	*Positive*	*Negative*	*Sensitivity (%)*
Pure gram positive	3	3	50	2	6	25
Pure gram negative	4	4	50	6	2	75
Mixed infection (gram positive and negative)	7	0	100	6	1	85
Candida	0	1	—	1	0	—
Mycoplasma	0	1	—	1	0	—
Uninfected	4	38	—	4	38	—
Total	18	47		20	45	

LAL, *Limulus* amebocyte lysate assay.

Table VI. Incidence of chorioamnionitis and endometritis according to Gram stain, *Limulus* amebocyte, and culture results

	Gram stain		*LAL*		*Culture*	
	Positive	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
Chorioamnionitis						
Present	10	7	8	9	11	6
Absent	8	40	12	36	12	36
Endometritis*						
Present	4	4	6	2	8	0
Absent	12	36	14	34	14	34

LAL, *Limulus* amebocyte lysate assay.

*For endometritis only 56 patients are considered. Nine patients with preterm labor were discharged undelivered (56 + 9 = 65).

amebocyte lysate assay have comparable diagnostic performance, but when used in combination there is remarkable improvement in sensitivity from 60.8% (Gram stain alone) to 95%. Furthermore, the negative predictive value increased from 80% (Gram stain alone) to 97% when both tests are negative.

Although the *Limulus* amebocyte lysate assay test would be expected to detect only gram-negative organisms, our data indicate that the test result was positive in 33% of pure gram-positive infections and also in cases of *Mycoplasma* or *Candida* intra-amniotic infection. Two possible explanations can be invoked. The infection could have included gram-negative organisms that were not grown in culture because of their fastidious nature or because they were not viable. In the latter case, free lipopolysaccharide released at the time of cell death could give a positive *Limulus* amebocyte lysate assay test result, although the organism may not grow in culture. An alternative explanation is that cross-reacting material induced gelation of the *Limulus* lysate. For example, peptidoglycans, a component of gram-positive bacteria, has been reported to produce a positive *Limulus* amebocyte lysate assay test result.[13] A similar observation has been made with mannan, a carbohydrate molecule isolated from *Candida* species.[14] Although the concept of cross-reactivity may be appealing because of its potential for broadening the spectrum of detectable organisms, this argument can also be spurious. The amount of peptidoglycans required to induce a positive *Limulus* amebocyte lysate assay test result is 400,000 times greater than that of lipopolysaccharide. In other words, contamination of the preparation with as little as 0.00025% of lipopolysaccharide could account for this finding. Such a minute degree of contamination cannot be ruled out in any "purified" material at present.[11]

Amniotic fluid culture has been used as the "gold standard" for the diagnosis of intra-amniotic infection in this study. Thus, one patient with a positive Gram stain and *Limulus* amebocyte lysate assay but a negative culture has been considered to have a false positive result for both techniques. This patient had severe pathologic chorioamnionitis and delivered a premature infant with subsequent negative neonatal cultures. Whether this case represents a false negative bacteriology culture is impossible to ascertain. The dilemma of the selection of an optimal standard is not a new problem in clinical diagnostic testing. Although overt clinical chorioamnionitis could be used as a standard, this would seem inappropriate because the goal of diagnostic testing is the early detection of infection before overt clinical disease.

Our findings that a positive amniotic fluid culture is associated with postpartum endometritis confirm pre-

vious observations of other investigators. Not surprisingly, a positive *Limulus* amebocyte lysate assay correlated with the presence of postpartum endometritis. We were puzzled by the lack of correlation between a positive *Limulus* amebocyte lysate assay and clinical chorioamnionitis and a positive Gram stain and postpartum endometritis. The former observation is probably because the *Limulus* amebocyte lysate assay detects primarily gram-negative bacteria. Indeed, it has a false negative rate of 50% in pure gram-positive infections occurring in patients who are at risk for clinical chorioamnionitis. The latter finding can be attributed to aggressive antibiotic therapy for all patients with a positive Gram stain for bacteria because this approach reduces postpartum endometritis.

Of practical importance is that the *Limulus* amebocyte lysate assay test is inexpensive (reagent cost is approximately $3), rapid, simple, and commercially available.

The detection of lipopolysaccharide in amniotic fluid has important scientific implications because endotoxins are a group of molecules with important biologic effects. Among their properties are the stimulation of prostaglandin release from macrophages and monocytes[15] and also the release of tissue factor from mononuclear cells.[16] These effects could play a role in the onset of labor or the activation of the hemostatic system in the setting of chorioamnionitis.

We conclude that the *Limulus* amebocyte lysate test is a sensitive, inexpensive, rapid, and simple technique for the detection of gram-negative bacteria in amniotic fluid that when used in conjunction with the Gram stain can significantly improve the detection of subclinical intra-amniotic infections.

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Maternal serum α-fetoprotein screening

III. Pitfalls in evaluating black gravid women

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Maternal serum α-fetoprotein levels are higher in black women. Misinterpretation of maternal serum α-fetoprotein screening results can subject black gravid women to unnecessary invasive diagnostic procedures and their calculable risks. Screening errors for black women can result from the use of normative data bases established with maternal serum samples drawn from other racial groups or the use of such data bases in conjunction with a published correction factor. Because the incidence of open neural tube defects is lower for blacks than for others, excessive false positive results for blacks (estimated to be 8817 to 28,215 cases annually) would be a pernicious misapplication of maternal serum α-fetoprotein screening. We address the problem outlined above and recommend independently developed, valid, normative data bases. (AM J OBSTET GYNECOL 1987;157:820-2.)

Key words: α-Fetoprotein, pitfalls, black gravid women

It is a physiologic fact that maternal serum α-fetoprotein levels are higher in black gravid women* than in other racial groups.[1-3] Maternal serum α-fetoprotein screening of blacks according to a normative data base established with blood samples drawn predominantly from other racial groups will subject blacks to an increased rate of false positive results. Because maternal serum α-fetoprotein levels are clinically significant in the high range[4] and may be so in the low range,[5] there exists a high probability of erroneous interpretation of maternal serum α-fetoprotein levels for blacks.

In reporting on a Connecticut maternal serum α-fetoprotein screening program, Baumgarten,[3] indicated that between 1981 and 1985, black gravid women demonstrated elevated maternal serum α-fetoprotein levels at more than double the rate (9.5%) of white gravid women (4.4%). This author indicates that maternal serum α-fetoprotein concentrations appear to be distributed differently between black and white racial groups and a higher multiple of the median may be required to exclude (from invasive diagnostic procedures) a similar percentage of blacks as whites in screening for open neural tube defects. Baumgarten points out that misinterpretation of data can follow omission of race as a factor in the assessment of maternal serum α-fetoprotein levels.

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**Blacks are defined in this study as persons who have designated themselves as such. Additional subgroup evaluation is underway.*

Table I. Racial differences* in median maternal serum α-fetoprotein levels

Gestation (wk)†	No. of Patients		% Difference of medians (black − white) / white
	Black	White	
15	295	761	16.6
16	568	1984	21.3
17	437	1549	21.6
18	337	827	2.8
19	203	396	17.6
20	114	180	−1.5

*These differences are based on median maternal serum α-fetoprotein levels without maternal weight adjustment. We applied three methods of maternal weight adjustment[7] that resulted in increased percentage differences between black and white median maternal serum α-fetoprotein levels.

†The mean gestational age for whites = 16.8 weeks; the mean gestational age for blacks = 17.0 weeks.

In comparing maternal serum α-fetoprotein levels between blacks and other racial groups, others have found that on the average blacks demonstrated a 10% increased level in weeks 14 to 22 of gestation.[1,2] Based on these findings and in an effort to correctly interpret maternal serum α-fetoprotein levels in black gravid women, the use of a 10% uniform correction factor (for gestational weeks 14 to 22) has been recommended to be applied to maternal serum α-fetoprotein levels in black gravid women.

Analysis of data

We have evaluated data (both our own and currently available data in the literature) comparing racial dif-

Table II. Projected maternal serum α-fetoprotein interpretations in the total black pregnant population in the United States (based on data generated from this study)

*Maternal serum α-fetoprotein interpretation**	*Method 1: use of black maternal serum α-fetoprotein medians*	*Method 2: use of white maternal serum α-fetoprotein medians*	*Method 3: use of 10% adjusted black values with white maternal serum α-fetoprotein medians*
Within normative range	547,830	519,615	539,013
Borderline elevation	22,336 (3.8%)	35,268 (6%)	25,275 (4.3%)
Significant elevation	17,634 (3.0%)	32,917 (5.6%)	23,512 (4.0%)
Total	587,800†	587,800	587,800

*The maternal serum α-fetoprotein interpretation is based on patient-specific risks.[4] Data presented pertain to maternal serum α-fetoprotein screening for structural malformations. Effect of race on Down's syndrome screening is under investigation.

†Total number of black births in the United States.[8]

ferences in levels of maternal serum α-fetoprotein. In a report from Crandall et al.,[1] individual weekly differences varied from as low as −4% to as high as 20%. Similarly, data from Baumgarten[3] demonstrate differences varying from 1% to 22%. Although a statistical analysis of available data might reveal an average 10% racial differential, careful evaluation of individual weekly differences indicates that the application of a uniform correction factor to maternal serum α-fetoprotein levels in blacks may indeed be inaccurate.

We have compared maternal serum α-fetoprotein levels between 5697 unaffected white and 1954 unaffected black pregnancies. Table I confirms previous observations that the percent difference of maternal serum α-fetoprotein medians between black and white gravid women varies with each week of gestation. However, what has not been reported previously, is that the variances of the black and white maternal serum α-fetoprotein distributions (based on log multiple of the median*) differ significantly.† If it is valid to interpret black maternal serum α-fetoprotein data by use of a 10% correction factor, these two distributions should be coincident. The significant difference in variance invalidates the use of this correction factor. Of additional interest is our observation that data presented by Baumgarten[3] (when analyzed by us) demonstrate a similar significant difference in variance between black and white maternal serum α-fetoprotein distributions.

Table II contrasts three methods of evaluating maternal serum α-fetoprotein values in black gravid women. Use of method 1 yields the lowest number of false positive results. With method 2, 70.6% more black gravid women will be informed of elevated maternal serum α-fetoprotein levels, indicating increased risk and counseling for further testing when white medians are used. With method 3, 22.1% more black gravid women will be counseled for increased risk when a 10% adjusted black value is used. With a valid data base (method 1) for all black gravid women in the United States (based on data generated in this study), false positive interpretations of maternal serum α-fetoprotein values would be reduced by 28,215 cases annually in comparison with method 2 and by 8817 cases annually in comparison with method 3.

*We note that multiple of the median as a statistical parameter of measurement is appropriate, but multiples of the median should not be used for reporting to clinicians or patients.

†The F statistic demonstrated significance of difference in variance ($p < 0.01$).

Comment

When a laboratory has screened a sufficient number of maternal serum α-fetoprotein samples to accumulate a significant sampling of black gravid women, two facts become apparent: (1) the mean of the distribution of maternal serum α-fetoprotein levels in black gravid women is significantly higher than that in other gravid women and (2) the dispersion of the levels in black gravid women is greater than in others. If either of these facts is not taken into account in evaluating maternal serum α-fetoprotein concentrations in black gravid women, black women will be subjected to unnecessary anxiety, expense, inconvenience, and potential maternal or fetal harm. To be spared this adverse clinical experience, it is necessary to evaluate maternal serum α-fetoprotein concentrations in blacks on the basis of a distribution of maternal serum α-fetoprotein levels established in a suitable population of black gravid women.

Because several investigators have reported higher concentrations of maternal serum α-fetoprotein levels in black gravid women, screening laboratories have begun to use a uniform 10% adjustment factor in interpreting black maternal serum α-fetoprotein levels. However, it is clear that although maternal serum α-fetoprotein levels are higher in black gravid women, the amount of difference between black gravid women and others is by no means consistent through successive weeks of gestation. Consequently, the application of any uniform adjustment factor is at best an approximation to physiologic reality and at worst a contribution to false positive findings.

Only when maternal serum α-fetoprotein screening is conducted on an appropriate scale[6] by a competent laboratory will the accumulation of maternal serum α-fetoprotein samples from black gravid women permit the development and use of a normative data base suitable to the population screened. This, in turn, will allow blacks to receive patient-specific risk reports based on relevant normative data.[4]

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Amniotic fluid α-fetoprotein levels and pregnancy outcome

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Elevated and low levels of maternal serum α-fetoprotein in the midtrimester of pregnancy have been linked with adverse events in later gestation, such as fetal and neonatal deaths, chromosomal abnormalities, and low birth weight infants. It is not known if this same association with poor pregnancy outcome is also true of amniotic fluid α-fetoprotein. In this study, α-fetoprotein was obtained from the fluid of 1060 women undergoing genetic amniocentesis for advanced maternal age. Poor pregnancy outcome was defined as (1) a fetal or neonatal death, (2) preterm delivery, or (3) low birth weight infants. Amniotic fluid α-fetoprotein was compared to each type of adverse outcome. No significant association with a poor pregnancy outcome in later gestation was noted. Although serum α-fetoprotein in the midtrimester of pregnancy may relate to certain poor outcomes in later gestation, midtrimester amniotic fluid α-fetoprotein offers no predictive value for the course of events in later gestation. (Am J Obstet Gynecol 1987;157:822-5.)

Key words: Amniotic, α-fetoprotein, pregnancy outcome

Elevated α-fetoprotein levels in maternal serum and amniotic fluid from the midtrimester of pregnancy are known to be associated with the presence of certain fetal anomalies, such as open neural tube defects, fetal abdominal wall defects, and cystic hygromas.[1] Recently the opposite end of the α-fetoprotein–screening spectrum, the low serum level, has been associated with chromosomal abnormalities,[2] and amniotic fluid α-fetoprotein levels have been found to be decreased in fetuses with Down's syndrome.[3]

In addition to aiding in the detection of fetal defects, abnormal maternal serum α-fetoprotein levels have been linked with adverse events occurring in later gestation. Elevated serum levels have been noted with fetal deaths, low birth weight infants, and neonatal deaths,[4] and low levels of maternal serum α-fetoprotein have been associated with an increased likelihood of a second-trimester fetal loss.[5] It has not been ascertained whether amniotic fluid α-fetoprotein levels in the midtrimester of pregnancy, like its serum counterpart, can predict subsequent adverse outcomes later in gestation. To address this question, our genetic amniocentesis data were reviewed retrospectively for a 2-year period to determine if a correlation existed between amniotic

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fluid α-fetoprotein values and subsequent adverse pregnancy outcomes.

Material and methods

The records of patients referred for prenatal diagnosis to the Laboratory of Medical Genetics at the University of Alabama at Birmingham from January 1, 1983, through December 31, 1984, were reviewed retrospectively. The prenatal diagnosis program is a collaborative effort between the Department of Obstetrics and Gynecology, which provides diagnostic ultrasonographic and amniocentesis services, and the Laboratory of Medical Genetics, which provides genetic counseling and laboratory services. The patient population consisted of private referrals from physicians in the southeastern United States, primarily from Alabama, Mississippi, Tennessee, Georgia, and Florida. The patients referred were primarily white (85%) and 93% were private referrals. Only 7% of patients seen were indigent referrals from county health departments in the state of Alabama.

Each patient underwent genetic counseling by a clinical geneticist before undergoing amniocentesis. Amniotic fluid α-fetoprotein determination was offered in addition to fetal karyotyping on the amniotic fluid samples obtained. Before each amniocentesis, an ultrasonographic examination was performed to document fetal viability, assess fetal anatomy, confirm gestational age, detect multiple gestation, and choose the amniocentesis site. Transplacental puncture was avoided whenever possible. The amniocentesis was performed by standard procedure with a 22-gauge spinal needle. Cytogenetic studies were performed at the Laboratory of Medical Genetics according to standard technique. All amniotic fluid α-fetoprotein samples were analyzed by the same reference laboratory (Aubrey Milunsky, Boston, Massachusetts), and determinations were performed by rocket electroimmunodiffusion, for which the normal range at each second trimester week have been published.[6]

Six months after the amniocentesis was performed a letter requesting follow-up data on each patient was mailed to each referring physician or county health department. The letter consisted of a series of questions asking whether any complications occurred immediately after the amniocentesis or later during the course of pregnancy. The date of birth, the infant's sex, birth weight, gestational age at birth, type of delivery, and whether any complications occurred at delivery or during the neonatal course were also asked in the questionnaire. If no response was obtained to the letter, a phone call was made to the physician's office or health department to obtain the requested information. If accurate follow-up data were still missing, phone calls were made to the patient's residence.

Table I. Mean α-fetoprotein levels at week of gestation studied with 95% confidence intervals

Week of gestation	*α-Fetoprotein (mg/dl)*
15	1.03 ± 0.070
16	1.03 ± 0.028
17	0.97 ± 0.039
18	0.85 ± 0.067
19	0.74 ± 0.072
20	0.77 ± 0.224

This study was restricted to patients who were undergoing the procedure because of advanced maternal age (≥35 years at delivery) and who had a singleton pregnancy between 15 and 20 weeks' gestation. Only patients with no serious medical problems and who underwent the procedure at our institution and by our physicians were included. Any patients who had a nonviable or structurally abnormal fetus at the time of the ultrasonographic examination or who later were found to have fetal chromosomal abnormality were excluded. Those patients who did not have both fetal karyotyping and α-fetoprotein determination of the amniotic fluid drawn at amniocentesis or complete follow-up information after delivery were excluded.

All records that met the criteria for inclusion in this study were reviewed retrospectively. The patient's age, race, parity, the color of the fluid at amniocentesis, and the best estimate of gestational age were recorded. If the menstrual dates were considered reliable and the average ultrasound measurements were within 10 days of the menstrual dates, the menstrual age was used to date the pregnancy. If there was a doubt as to the last menstrual period or if the ultrasonographically determined age varied by >10 days from the menstrual age, the gestation by ultrasound was used to date the pregnancy. The fetal karyotype and amniotic fluid α-fetoprotein values were recorded on each patient and then matched with the letter containing follow-up information from each pregnancy.

Poor pregnancy outcome was defined as: (1) fetal or neonatal death, (2) delivery before 37 completed weeks of gestation, or (3) delivery of an infant weighing ≤the tenth percentile for its expected gestation (low birth weight). Tenth percentile birth weights at each gestation were calculated from data published previously and determined from abortuses and infants delivering at sea level.[7] Because of the large number of private physicians participating in the prenatal care and delivery of these infants, we were unable to include the prenatal management, mode of delivery, Apgar scores, or presence of fetal distress into consideration for factors predicting adverse pregnancy outcome.

Table II. Correlation of poor outcome with amniotic fluid α-fetoprotein level

Poor outcome	*n*	*Least-square mean ± SE (mg/dl)*	*α-Fetoprotein p value**
Preterm delivery			
Yes	66	0.93 ± 0.04	0.27 (NS)
No	994	0.98 ± 0.01	
Fetal or neonatal death			
Yes	21	0.85 ± 0.07	0.08 (NS)
No	1039	0.98 ± 0.01	
Low birth weight			
Yes	34	0.96 ± 0.06	0.76 (NS)
No	1026	0.97 ± 0.01	

*By analysis of covariance.

There were 1060 women with singleton pregnancies and complete follow-up information who underwent genetic amniocentesis for advanced maternal age at our institution from January 1, 1983, through December 31, 1984. There were 159 primigravid and 901 multiparous women. Nine hundred one women were white (85%) and the median maternal age of the study group was 36 years.

The Student t test and χ^2 analyses were used to correlate maternal factors and results with pregnancy outcomes. A p value ≤ 0.05 was considered significant. Standard analysis of variance and covariance analysis of variance techniques were used to determine any significant maternal age and α-fetoprotein relationship for the different complications at birth. Duncan's multiple range test was used for all multiple comparisons. The Kolmogorov-Schmirnoff test of goodness of fit was used to determine if the data constituted a random sample from a normal population.

Results

No statistically significant differences were noted between primigravid and multiparous women and between different racial groups in the study population when each was compared to the level of amniotic fluid α-fetoprotein. Four hundred forty-four women in this study were ≤36 years of age and 152 women were ≥39 years of age. There was no difference in amniotic fluid α-fetoprotein levels when compared to these extremes of maternal age in this study. One thousand eight patients had α-fetoprotein values ±2 SD within the mean at each gestational age. Forty-three were >3 SD above the mean at any given gestational age. Two patients had α-fetoprotein values as high as 5 SD above the mean. No α-fetoprotein values fell as low as 2 SD below the mean for a given gestational age.

Sixty-six (6%) women were delivered before 37 weeks' gestation, 34 (3%) had low birth weight infants, and 21 (2%) had fetal or neonatal deaths. The fetal deaths were the result of unexplained causes and the neonatal deaths were primarily caused by extreme prematurity. Eleven patients had losses in the second trimester and 10 deaths were near term.

Two different statistical analyses were used to compare amniotic fluid α-fetoprotein with each type of poor pregnancy outcome. Table I illustrates the 95% confidence intervals for amniotic α-fetoprotein at each week of gestation. Table II depicts the statistical lack of correlation by analysis of covariance using week of sampling as the covariant. The least-square mean α-fetoprotein levels were lower in those patients who later were found to have a fetal or neonatal death but not in those patients delivering low birth weight infants or preterm infants. Although this finding is an interesting trend, it did not reach statistical significance. Table III shows the wide variation in amniotic fluid α-fetoprotein levels with each type of poor outcome. Amniotic fluid α-fetoprotein levels could not be used to predict a later adverse outcome because of their wide overlapping with values in patients with normal outcomes.

Thirty-six patients (3%) had discolored fluid at amniocentesis. Our laboratory does not routinely test for fetal hemoglobin unless an α-fetoprotein values exceeds 3 SD above the mean. One patient with discolored fluid had a value above 3 SD but did not have fetal hemoglobin in the amniotic fluid sample. Discolored fluid was not statistically associated with a poor pregnancy outcome.

Comment

The physiology of amniotic fluid α-fetoprotein has been described extensively.[8] During normal pregnancy amniotic fluid α-fetoprotein follows a decreasing pattern similar to that of fetal serum and is contributed to normal amniotic fluid by fetal urination. In this study, 1060 women undergoing genetic amniocentesis for advanced maternal age were offered and chose to have α-fetoprotein determination on the amniotic fluid samples obtained. Fetal structural abnormalities and patients with medical problems were excluded from analysis of the final data. The amniotic α-fetoprotein values

Table III. Distribution of amniotic fluid α-fetoprotein values around the 95% confidence interval with each type of poor outcome

Poor outcome	*No. (%) patients below 95% confidence interval**	*No. (%) patients above upper 95% confidence interval**	*p Value*
Preterm delivery			
Yes	30 (46.9)	26 (40.6)	0.42 (NS)
No	531 (53.6)	376 (37.9)	
Fetal or neonatal death			
Yes	14 (66.7)	4 (19.1)	0.17 (NS)
No	547 (52.9)	398 (38.5)	
Low birth weight			
Yes	22 (64.7)	8 (23.5)	0.20 (NS)
No	539 (52.8)	394 (38.6)	

*Each 95% confidence interval has been calculated by gestational age at time of amniocentesis.

were in a range similar to what has been reported in other populations studied.[9]

Our patients were primarily white middle-class women from the southeastern United States. They were private referrals and all were in excellent health. The outcomes of their pregnancies were generally good and were similar to what has been published about other populations in the advanced maternal age range.[10] Although low and elevated serum α-fetoprotein levels have been linked to adverse outcomes in later gestation, no one to date has published a study comparing amniotic fluid α-fetoprotein levels with later pregnancy outcomes. However, we could find no statistical correlation between amniotic α-fetoprotein values and a later poor pregnancy outcome. In our study population, patients who later had a poor pregnancy outcome had α-fetoprotein values within the normal range and thus too closely overlapped those with normal outcomes to be of any predictive value.

If a correlation with adverse events in later pregnancy is found with abnormal levels of maternal serum α-fetoprotein, then why is there no such finding with amniotic fluid α-fetoprotein levels? It has not been clearly defined yet how α-fetoprotein passes from the fetal compartment into the maternal serum. It has been suggested[11] that the placenta or amnion regulates this protein interchange. Thus a genetically abnormal or physiologically compromised placenta in the midtrimester of pregnancy would cause abnormal α-fetoprotein levels in maternal serum. Because α-fetoprotein enters the amniotic fluid presumably by fetal urination after the fourteenth week of gestation, placental insufficiency from whatever cause would have no bearing on the amount of α-fetoprotein in the amniotic fluid with α-fetoprotein levels remaining within the normal range.

In summary, amniotic fluid α-fetoprotein in the midtrimester of pregnancy has no correlation with later preterm births, fetal or neonatal deaths, or low birth weight infants. A normal α-fetoprotein value obtained at genetic amniocentesis is reassuring that no major fetal structural defect associated with elevated amniotic fluid α-fetoprotein is present at the time of sampling but offers no predictive value to the course of events for that pregnancy in later gestation.

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Cord serum bromide concentration: Variation and lack of association with pregnancy outcome

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Excessive maternal bromide exposure during pregnancy from drugs and occupation have been reported to have adverse effects on the fetus and newborn, including central nervous system depression at birth and possible teratogenicity. To define further the fetal bromide exposure during pregnancy, we determined the cord serum bromide concentration in 1267 newborn babies born in Rochester, New York, during the 6-month period from January 1, 1984 to June 30, 1984. There was a normal distribution of the cord serum bromide concentration values ($\bar{X} \pm SD = 8.6 \pm 2.6$ mg/L; range 3.1 to 28.5 mg/L). The highest concentration was still significantly below the minimal bromide concentration associated with toxic effects (720 mg/L). There was no association between the cord serum bromide concentration and indices of fetal health including Apgar scores, presence of congenital malformations, and neonatal disposition. No mothers were taking significant amounts of bromide-containing drugs during pregnancy. The second highest cord serum bromide concentration (21.4 mg/L) was in a woman who was an amateur photographer and developed her own film, which required her to use chemicals containing bromide. Our results indicate that excessive fetal bromide exposure is rare and probably occurs only in the setting of maternal use of bromide-containing drugs or occupational exposure during pregnancy. (Am J Obstet Gynecol 1987;157:826-30.)

Key words: Bromide, cord blood, pregnancy, outcome

Bromide is normally present in low concentrations in water and many foods.[1] Several reports of excessive maternal bromide exposure during pregnancy have indicated adverse effects on the fetus and newborn infant.[2-6] These maternal exposures were from bromide-containing drugs and occupational exposure to bromide-containing salts in the photographic industry. To study the issue of fetal bromide exposure during pregnancy further, we determined the cord serum bromide concentration in a cohort of newborn infants born at a tertiary care hospital to determine the variation in concentrations and whether there was any correlation between the cord serum bromide concentration and pregnancy outcome. Performing this study in Rochester, New York, where the largest photographic manufacturing and developing company in the United States is located (Eastman Kodak), provides the greatest possibility of detecting occupational exposure to bromides causing elevated cord serum bromide concentration.

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Methods

Specimen collection. Five milliliters of cord blood was drawn from babies born at Strong Memorial Hospital, Rochester, New York, between January 1, 1984, and June 30, 1984. The cord serum was recovered and placed in a plastic tube and stored at −70° C until analyzed.

We also obtained maternal blood specimens at the time of delivery in 20 obstetric patients and the corresponding cord blood specimen from their baby, so that bromide concentrations in each could be determined and compared.

Bromide analysis. The bromide concentration was determined in serum ultrafiltrate of each cord blood and maternal blood specimen by use of a previously described anion-exchange chromatographic method.[7] Serum ultrafiltrate was obtained by centrifugation of 1 ml of serum in an Amicon MPS micropartition system at 2000 × g for 25 minutes.

The chromatography system was a Varian 5020B with a UV-100 variable wavelength detector interfaced with a Varian 4270 integrator. A Whatman Partisil-10 SAX 250 by 4.6 mm inside diameter anion exchange column was used with 30 mmol/L KH2P04 mobile phase at a flow rate of 0.7 ml/min. The detection wavelength was 200 nm and the injection volume was 5 μl. Under these conditions the retention time of the bromide peak was about 11.5 minutes. The serum ultra-

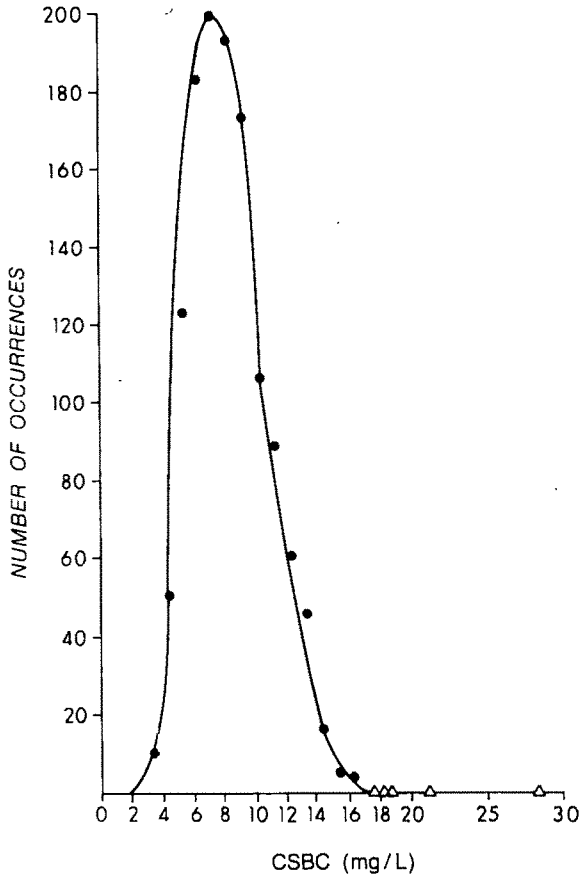


Fig. 1. Frequency distribution of cord serum bromide concentration *(CSBC)* in 1267 specimens. Each point represents the number of occurrences of cord serum bromide concentration between integral values of the concentration plotted at midinterval, in that the number of occurrences between 3 and 4 mg/L is plotted at 3.5 mg/L, the number of occurrences between 4 and 5 mg/L is plotted at 4.5 mg/L, and so forth. The *triangles* represent individual values of the five highest cord serum bromide concentrations.

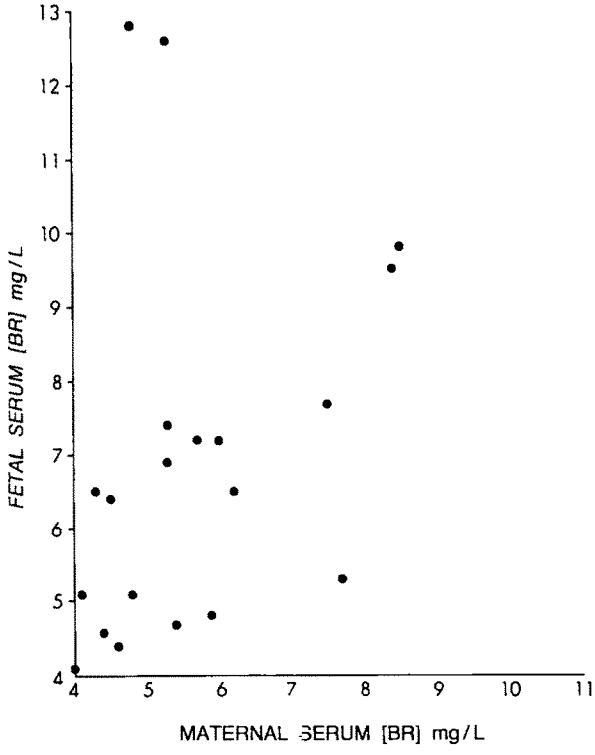


Fig. 2. Scatter plot of maternal serum versus fetal serum bromide concentration *([BR])*. There was general agreement between the two values, with the fetal serum bromide concentration level tending to be slightly greater. There is no obvious explanation for the two points that indicate a significantly higher fetal than maternal serum bromide concentration. The correlation coefficient is 0.33, and is 0.71 when the two discrepant values are excluded.

filtrate concentration was determined by the area under the curve as compared with those of known bromide standards for which absorbance was linear from 1 to 1000 mg/L.

Because serum is 93% water, the cord serum bromide concentration is 0.93 × the cord serum ultrafiltrate bromide concentration.

Data collection and analysis. Data about every obstetric admission and delivery at Strong Memorial Hospital are kept at The Perinatal Data Registry of the Rochester Regional Perinatal Center. The following information for each pregnancy and baby was abstracted from the registry: race of the mother, presence of hypertension in the mother, radiation exposure during pregnancy, drug exposure during pregnancy, maternal occupational history, neonatal outcome (stillborn, death during perinatal period, or live born and discharged home), resuscitation required after delivery, Apgar score at 1 minute, Apgar score at 5 minutes, presence of congenital malformation(s), newborn temperature, newborn respiration rate, newborn pulse, and newborn weight. Mothers were asked about drug usage during pregnancy but not specifically about bromide-containing drugs. The relationship of each of these variables to the cord serum bromide concentration was tested by scatter plots, correlation coefficients, and, for categorical variables, one-way analysis of variance. The cord serum bromide concentration and maternal bromide serum concentration at delivery were compared by determining the absolute percent agreement [(lower of the two values/higher of the two values) × 100%] and by determining the correlation coefficient between them.

Results

During the 6-month period, cord blood specimens for determination of cord serum bromide concentration were available from 1267 of the 1591 babies born at Strong Memorial Hospital. The range of the con-

Table I. Cases of maternal bromide exposure causing adverse fetal effects

Case	*Patient's age*	*Neonatal history*	*Maternal exposure*	*Bromide determination**
Mangurten and Kaye[2]	Newborn (43 wk)	Cyanosis, hypotonia, poor feeding, dysmorphic face	Worked in photographic laboratory	Baby's urine (day 14): 330 mg/L Mother's serum (day 18): 190 mg/L Baby's serum (day 18): 150 mg/L
Mangurten and Ban[3]	Newborn (37 wk)	Weak cry, poor suck, hypotonia	6 gm bromide per day	Mother's serum (day 5): 3100 mg/L Baby's serum (day 5): 2000 mg/L Baby's urine (day 5): 1660 mg/L
Pleasure and Blackburn[4]	Newborn	Hypotonia	Nervine tablets	Mother's serum (day 4): 2150 mg/L Baby's serum (day 5): 2420 mg/L
Finken and Robertson[5]	Newborn (39 wk)	Lethargy	Nervine tablets	Baby's serum (day 7): 3650 mg/L
Opitz et al.[6] (siblings)	a. 8 yr b. 7 yr	a. Growth deficiency b. Growth deficiency; congenital heart disease	Chronic ingestion of Bromo-Seltzer	Mother's serum: 1350 mg/L

*Normal values[2]: urine bromide concentration <2.5 mg/L; serum bromide concentration <20 mg/L.

centration was 3.1 to 28.5 mg/L, with a mean of 8.6 mg/L and a standard deviation of 2.6 mg/L. The standard error of the mean is therefore only 0.073 mg/L, and the 95% confidence interval around the mean is between 8.46 and 8.74 mg/L. The frequency distribution of the cord serum bromide concentrations is shown in Fig. 1. There was no significant deviation of this distribution from a normal distribution as measured by kurtosis or skewness.

Statistical testing of the cord serum bromide concentration against the 14 parameters mentioned above showed no significant findings. Noteworthy was that the mother of the baby with the second highest cord serum bromide concentration (21.4 mg/L) was an amateur photographer who developed her own photographs at home.

A scatter plot of the 20 paired maternal serum and cord serum values for bromide concentration is shown in Fig. 2. The two values tended to be similar, with the cord serum bromide concentration tending to be a little higher than the mother's serum bromide concentration. The absolute percent agreement between the two values was 78%. The correlation coefficient between these two measurements was 0.33; when the two discrepant values in the upper left corner were excluded, however, the correlation coefficient was 0.71.

Comment

At least four case reports of neonatal bromism secondary to maternal ingestion or exposure to bromide-containing compounds during pregnancy have been published (Table I).[2-5] The cardinal features in these babies were weakness and hypotonia. Maternal exposure in three cases was from the use of bromide-containing drugs during pregnancy and in one case from a presumed occupational exposure to bromide salts in the photographic developing industry. In addition, Opitz et al. described two siblings with significant postnatal growth deficiency who were born to a mother who had been taking the bromide-containing drug Bromo-Seltzer during both of these pregnancies (Table I).[6] (Bromo-Seltzer contained bromide before 1972 but no longer does.) One of the affected children also had congenital heart disease. The mother took no Bromo-Seltzer during the pregnancies of three other healthy siblings who were of normal stature. This observation raises the question of potential teratogenic effect of bromide exposure during pregnancy.

There are four possible sources of bromide exposure that could lead to systemic absorption and accumulation: drugs, occupational exposure, food, and water. Bromide is found in very small quantities in food and water. To the best of our knowledge, no cases of human bromism have been reported from the ingestion of either food or water of high bromide content.

The use of bromide-containing drugs in the United States has significantly decreased in the past 25 years, and there are few, if any, prescription drugs that now contain significant amounts of bromide. Bromide formerly enjoyed widespread use as an anticonvulsant drug and in nonprescription sedatives.[8] However, bromide has a long biologic half-life (12 days), and thus

prolonged administration of bromide can lead to bromide toxicity. Bromide-containing drugs have therefore almost entirely been removed from the market.[9] Thus it is not surprising that in our population of over 1200 mothers we found none with a history of bromide-containing drug use.

There is little information on occupational exposure to bromides causing bromide accumulation in exposed individuals. There is one reported case of neonatal bromism from occupational exposure to bromides.[2] Mangurten and Kaye (case 1; Table I) reported a 25-year-old pregnant woman who, although denying the use of bromide-containing drugs, was found to have an elevated serum bromide level 18 days after the birth of her baby, who had clinical signs of neonatal bromism with significant unexplained hypotonia and generalized central nervous system depression. The baby had a serum bromide level of 150 mg/L on the eighteenth day of life and had a markedly elevated concentration of bromide in the urine. The mother's occupational history revealed that she had worked in a photographic laboratory until 5 weeks before delivery. She mixed photographic developing chemicals containing sodium bromide and potassium bromide on a daily basis. Those authors concluded that the occupational exposure to bromide-containing photographic compounds was the basis of asymptomatic "maternal bromism," which led to symptomatic neonatal bromism. Although it is not clear what the route of exposure was in her case, inhalation, skin absorption, or ingestion of chemical dusts or chemical solutions are all possibilities.

Bromide can penetrate human skin, although it does so at a very slow rate (0.09 to 0.16 mEq/cm^2/min).[10] This absorption rate is similar to those of sodium, potassium, and phosphate. These ions are absorbed to a significant extent through rabbit skin (3 to 8 mEq/cm^2/min), and to a modest extent through pig skin (0.5 to 5 mEq/cm^2/min). The finding that the second highest cord serum bromide concentration (21.4 mg/L) was in a mother who was an amateur photographer and developed her own films suggests that there can be systemic absorption of bromide from handling bromide-containing chemicals used in photographic development. It must be emphasized that while the cord serum bromide concentration in this baby was elevated compared with the mean of this population, it was significantly below what is considered to be a toxic level, namely >720 mg/L.[11]

Bromide is found in low concentrations in food, drinking water, and beverages. In The Netherlands the bromide concentration of vegetables is about 4 mg/kg, milk 3 mg/kg, meat 4 mg/kg, fish 7 mg/kg, and drinking water 0.1 mg/kg.[1] The daily intake of bromide by humans in The Netherlands during the summer of 1976 was 7.8 mg (range 3 to 15 mg) and during the winter of 1978 it was 7.6 mg (range 2 to 17 mg). With this average daily intake of bromide, and assuming that the individual weighs 70 kg, that the volume of distribution of bromide is 0.3 L/kg,[12] and that the bromide half-life is 12 days,[9] the steady-state serum bromide concentration is calculated to be 6.4 mg/L (see Appendix). This is in agreement with the values for mean cord serum bromide concentration and mean adult female serum bromide concentrations we found. These data suggest that the vast majority of bromide found in the average person comes from food, beverages, and water.

Our results indicate that there is some variation in the cord serum bromide concentration, but even the highest value of the more than 1200 specimens studied was significantly below the minimum toxic bromide concentration. The mean value of 8.6 mg/L is 1.2% of the minimum serum bromide concentration associated with toxicity. The large sample size of 1267 specimens gives a large power in the detection of small differences in the order of 10% of the mean value (±0.9 mg/L) or 50% of the standard deviation (±1.3 mg/L). Under the most extreme of these possibilities, the highest mean cord serum bromide concentration would still only be 9.9 mg/L (8.6 + 1.3 mg/L). No mothers appeared to be taking bromide-containing drugs and only one mother who was exposed to bromides from occupational exposure (amateur photographer). This would suggest that there is no fetal risk from bromide exposure in the baby born to a mother who is not taking bromide-containing drugs and who is not exposed to bromides from her occupation. In instances in which mothers are taking bromide-containing drugs or handle photographic developing chemicals during pregnancy, the pediatrician should keep in mind the possibility of bromide toxicity if perinatal problems are encountered.

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Appendix

Calculation of steady-state serum bromide concentration

V_d = volume of distribution of bromide
Cl = clearance of bromide
t½ = half-life of bromide
K_{el} = elimination constant of bromide
$[Br]_{ss}$ = steady-state serum bromide concentration
DI_{Br} = daily intake of bromide

A. Pharmacokinetic relationships

1. $Cl = V_d \times K_{el}$
2. $K_{el} = \frac{0.693}{t_{1/2}}$
3. $[Br]_{ss} = \frac{DI_{Br}}{Cl}$

B. Combining 1, 2, and 3; assume 70 kg individual; assume daily bromide intake in United States is similar to that in The Netherlands

$$[Br]_{ss} = DI_{Br} \times \frac{t_{1/2}}{0.693 \times V_d}$$

$DI_{Br} = \frac{7.7 \text{ mg}}{70 \text{ kg}}$ (reference 1)

t½ = 12 days (reference 9)

V_d = 0.3 L/kg (reference 12)

$[Br]_{ss}$ = 6.4 mg/L

Fetal-induced trauma as a cause of antepartum hemorrhage

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External trauma is a well-known cause of antepartum hemorrhage. These two cases demonstrate that fetal trauma may also be a cause of antepartum hemorrhage. Both patients complained of painful fetal movements, and real-time ultrasonography demonstrated that the fetus was striking the placental edge.
(Am J Obstet Gynecol 1987;157:830-1.)

Key words: Antepartum hemorrhage, ultrasonography

Trauma has long been known to be a cause of premature placental separation.[1] Such trauma includes blunt injury to the maternal abdomen, external cephalic version, and sudden uterine decompression. As the following two cases illustrate, it would seem that fetal trauma must be added to this list as a cause of antepartum hemorrhage.

Case reports

Case 1. A 24-year-old woman, gravida 4, para 2, came to the labor ward at 36 weeks' gestation as determined by dates and early ultrasound scan with genital tract bleeding. The bleeding had been preceded by painful fetal movements. She had an unremarkable past medical and obstetric history. The results of a cervical smear were negative. An examination showed that the abdomen was soft with a fundal height that was consistent with the dates. The fetus was a transverse lie with the head on the maternal right. An ultrasound scan was performed in the labor ward. The fetal biometry was consistent with the dates, and the estimated weight of the fetus was 3.1 kg. The placenta was noted to be anterior, with the lower edge just reaching the internal os (i.e., a Grade II placenta previa). A loop of cord and both fetal hands were noted to be near the cervix. During the scan, one of the fetus' hands was seen to strike the lower most edge of the placenta and actually indented the lower uterine segment. This occurred several times during the scan, and on each occasion the patient winced with pain and some blood was noted to run from the vagina. The patient was admitted to the hospital and the bleeding subsided spontaneously. At no time was any evidence of uterine contractions detected with external cardiotocography, nor was there any progressive cervical dilatation or evidence of ruptured membranes. The baby was delivered by cesarean section one week later. The patient's postoperative

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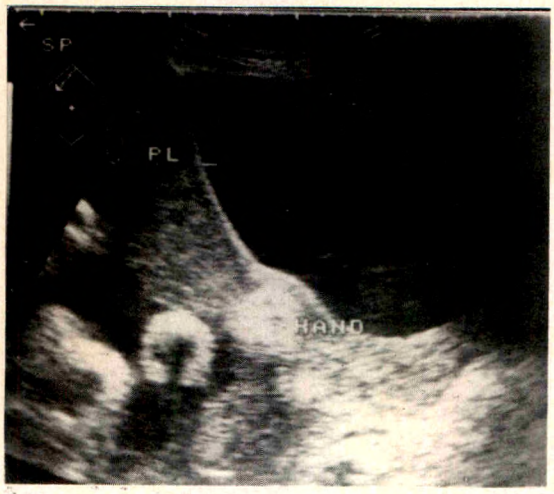


Fig. 1. Longitudinal scan shows the fetal hand striking the lower edge of the placenta.

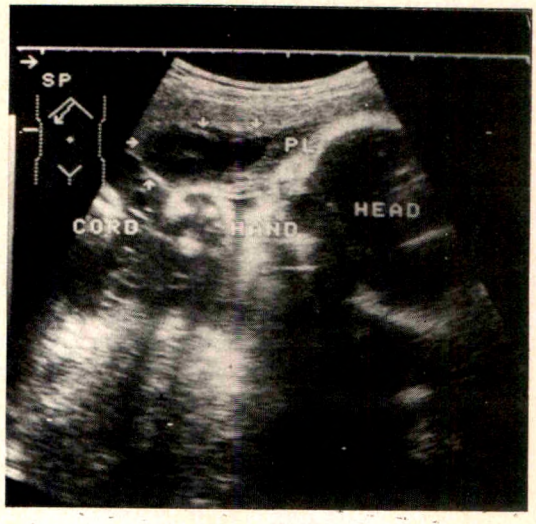


Fig. 2. Retromembranous clot in close proximity to the fetal hands.

course was uneventful, and she was discharged with her baby on the seventh postoperative day.

Fig. 1 is a longitudinal scan that shows the fetal hand striking the lower edge of the placenta.

Case 2. A 22-year-old primipara came to the labor ward at 34 weeks' amenorrhea as determined by dates and early ultrasonography. The patient had noticed that fetal movements had become painful, and some minutes later she noticed blood in the vagina. An examination showed that the fundal height was consistent with the dates, and the fetus was a cephalic presentation, right occipitoposterior. A very localized area of tenderness was noted over the right anterolateral portion of the uterus. The cervix was closed, and there was no evidence of leakage of amniotic fluid. External tocography failed to show any uterine activity. An immediate ultrasound scan was performed. The fetal biometry was consistent with the dates, and the estimated weight was 2.5 kg. The placenta was noted to be on the upper right posterolateral wall of the uterus. A small marginal hemorrhage (3.5 by 2.8 cm) was seen over the area of tenderness, clearly stripping up a portion of fetal membranes. Both fetal hands were seen to "punch" this area and caused the patient much pain. The results of a clotting screen were normal. The bleeding subsided spontaneously with no specific treatment, and she was allowed to go home after a few days. The patient returned 1 month later and had a spontaneous normal vaginal delivery.

Fig. 2 shows that the retromembranous clot was in close proximity to the fetal hands.

Comment

Antepartum hemorrhage has traditionally been divided into three types—abruptio placentae, placenta previa, and indeterminate antepartum hemorrhage. This latter group includes marginal hemorrhage from a normally sited placenta.[2] Bleeding from a placenta previa may follow local trauma such as coitus or vaginal examination. Case 1 illustrates that fetal trauma may also cause a low-lying placenta to bleed. Case 2 clearly illustrates that fetal trauma can also sometimes injure the margin of a normally situated placenta.

Both of these cases illustrate the usefulness of ultrasound examination in the accurate diagnosis of the cause of an antepartum hemorrhage. Furthermore, they illustrate the importance of attempting to scan such patients as soon as possible after admission to the hospital. Both patients complained of painful fetal movements followed by vaginal bleeding. The patients' complaint of pain also coincided exactly with the fetus' striking its placenta. In these two cases vaginal bleeding subsided spontaneously without specific treatment. However, had the bleeding not stopped and the fetal movements continued to be painful, it would seem reasonable to sedate the mother and thus sedate the fetus.

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A pregnant woman with a high level of naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii*

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A pregnant woman diagnosed as positive for immunoglobulin M antibodies to *Toxoplasma gondii* did not have a subsequent rise of specific immunoglobulin G antibodies until delivery. The antibodies were therefore considered as naturally occurring immunoglobulin M antibodies. The baby was completely normal without any clinical and serologic signs suggestive of congenital toxoplasmosis. (Am J Obstet Gynecol 1987;157:832-3.)

Key words: *Toxoplasma gondii*, pregnancy, natural immunoglobulin M, serodiagnosis

Infection with *Toxoplasma gondii* newly acquired during pregnancy is well known to cause congenital toxoplasmosis in infants. Immunologic diagnosis of toxoplasmosis for pregnant women is therefore in an important position as one of the TORCH (*Toxoplasma*, rubella virus, cytomegalovirus, and herpes simplex virus) group. We report on a case in Japan in which a pregnant woman was erroneously suspected of recent infection with this teratogenic parasite because of a high level of naturally occurring immunoglobulin M antibodies directed against *Toxoplasma*.

Case report

A 31-year-old woman was first examined for pregnancy at a maternity clinic in January 1985. At this time, she presented at 8 weeks' gestation and was found positive for *Toxoplasma* antibodies by a routine latex agglutination test. Although no clinical signs and symptoms were observed in the mother and her fetus, she came to the Department of Gynecology and Obstetrics in our university hospital at 14 weeks and asked for closer examination simply because of fear of the result of the antibody test.

In my laboratory, her serum was initially tested for *Toxoplasma* antibodies by the latex agglutination test, and the titer was 1:256, which is consistent with the previous result. When enzyme-linked immunosorbent assay[1] was used, the serum was only positive for immunoglobulin M class antibodies at an enzyme-linked immunosorbent assay value of 1.046 but was negative for immunoglobulin G antibodies at an enzyme-linked immunosorbent assay value of 0.013. It strongly suggested the recent acquisition of *Toxoplasma* infection accompanied by the risk of fetal infection in her uterus. Fractionation of immunoglobulin G and immunoglobulin M from the serum on sucrose density gradient also resulted in the presence of immunoglobulin M antibodies and absence of immunoglobulin G antibodies. This was further confirmed by the conventional indirect fluorescent antibody test in which the immunoglobulin M antibody titer was found to be 1:80.

At 16 weeks' gestation, the woman was again examined for antibodies. As opposed to the common immunologic knowledge of the course of immunoglobulin G and immunoglobulin M antibodies after infection, the enzyme-linked immunosorbent assay value of immunoglobulin G antibodies was still negligible and that of immunoglobulin M antibodies was still high. A similar situation was again observed at 23 weeks and at delivery. Based on this antibody course, the previous result of latex agglutination test at 8 weeks also seems to be attributed merely to the presence of immunoglobulin M. Serum obtained from the umbilical cord was negative for immunoglobulin M antibodies to *Toxoplasma* at an enzyme-linked immunosorbent assay value of 0.037, and the total amount of immunoglobulin M was less than 20 mg/dl, a borderline value for intrauterine infection. The fetus grew normally during gestation, and the baby subsequently had no clinical signs related to generalized infection, central nervous system abnormalities, or eye lesions, at least during the first 3 months of life.

Comment

The continued high antibody level of immunoglobulin M without a rise in the immunoglobulin G antibody level was unlikely to be explained by a general immune response to the infection. Our recent study on immunoglobulin M antibodies to *Toxoplasma* in a swine population[2] revealed that all persons inherently had a variety of immunoglobulin M antibody levels even without infection, and such antibodies were therefore considered naturally occurring immunoglobulin M antibodies. A survey of a human population negative for immunoglobulin G antibodies also showed the presence of immunoglobulin M antibodies in varying degrees.

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If a woman who has a high level of naturally occurring immunoglobulin M antibodies becomes pregnant, she may be probably suspected of recent acquisition of *Toxoplasma* infection, even though neither she nor the baby is infected at least during gestation, as was seen in the present case. The characteristics of naturally occurring immunoglobulin M antibodies compared with immunoglobulin M antibodies raised by infection are now under investigation by means of Western blotting analyses, especially to eliminate such false-positive results for recent *Toxoplasma* infection in pregnant women.

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The umbilical cord twist: Origin, direction, and relevance

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The purpose of this study was to evaluate the origin, direction, and relevance of the umbilical cord twist. We initially hypothesized that the direction of the helix or twist of the human umbilical cord at birth correlated with the eventual handedness of the child. Among 2801 singleton placentas in this study, only 5% had no twist, and the left twist outnumbered the right twist by 7 to 1, a ratio that is strikingly similar to the predominance of right-handed persons to non-right-handed persons in the general population. Forty-five 3- and 4-year-old children with previously documented cord twists were evaluated with respect to hand preference and performance. The direction of the cord twist was independent of the handedness of the child as well as the mother. We have documented an increased incidence of absent twist and right twist in association with single umbilical artery, suggesting that the impetus for the cord twist is independent on hemodynamic forces in the umbilical cord itself. We further document an increased incidence of absent twist among intrauterine fetal deaths and twins, suggesting that decreased fetal movement can impede the forces leading to normal twisting of the umbilical cord. Absence of cord twist may be associated with adverse prognosis. (Am J Obstet Gynecol 1987;157:833-8.)

Key words: Umbilical cord twist, placenta, handedness, asymmetry

The spiral course of the umbilical cord vessels was recorded by Berengarius in 1521.[1] The twist, or more properly the helix of the umbilical cord (Fig. 1), which has been observed as early as 42 days' gestation, is well established by 9 weeks.[2, 3] The number of twists present during the first trimester is roughly the same as that seen in term cords (most often between 0 and 40, but up to 380), indicating that the cord lengthens by an increase in the pitch between each turn of its helix rather than an increase in the number of turns.[1, 4, 5] Although the cause of the twist of the cord is unknown, Leonardo da Vinci observed in the fifteenth century that "the length of the umbilical cord is equal to the length of the child in every stage of its age,"[1] and more recent research suggests that the length of the cord at birth is influenced by intrauterine fetal activity and therefore correlates with fetal well-being. The fact that animals that are normally constrained within an elongated uterine cavity throughout gestation[5] and humans whose intrauterine activity has been abnormally restricted because of intrinsic defects in development or extrinsic constraint[6-10] often have short umbilical cords that lack normal twists suggests that not only the length of the cord at birth but also the twist of the cord depends on intrauterine fetal activity.

In 1600 Fabricius demonstrated with illustrations that both right, or clockwise, and left, or counterclock-

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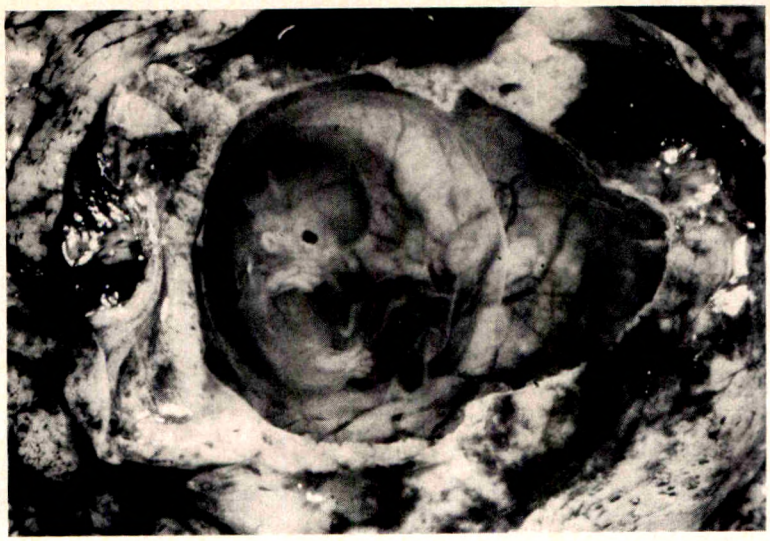


Fig. 1. The umbilical cord twist is well established by 9 weeks' gestation. Note the left twist of the umbilical cord in this 8-week specimen.

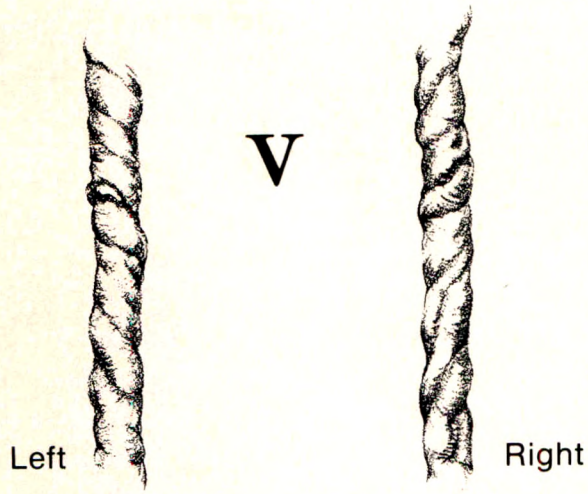


Fig. 2. Direction of the umbilical cord helix: the anterior portion of a left helix parallels the left-hand limb of a letter V, whereas the anterior portion of a right helix parallels the left-hand limb of a letter V.

wise, helices of the umbilical cord occur.[1] If the umbilical cord twist were determined randomly, one would expect the incidence of the right twist to be roughly equal to that of the left twist. However, a long series of investigators have found that the majority of cords twist to the left.[1,3,4] Although the reason for this discrepancy remains unexplained, this disproportion is strikingly similar to the predominance of right-handed persons to non-right-handed persons in the general population.[11] Pande and Singh[12] found significant asymmetry in muscle and bone weight in the upper limbs of human fetuses, the weight being greater on the right side in eight out of nine specimens, suggesting that handedness could conceivably be determined at the time of the umbilical cord twist is being established. The aim of the present study was to determine whether the direction of the cord twist at birth correlated with the eventual handedness of the child and thus reflected the direction that the fetus actively rotated in utero.

Subjects and methods

The placentas of all infants delivered at or transferred to our institution are examined routinely by a single pathologist (K. B.). Microscopic examination and special studies are performed when indicated. Since April 1982 we have recorded the direction of the twist for each umbilical cord. The direction of the helix is established as follows (Fig. 2): If a helix is held vertically the course of the anterior portion of a left helix will parallel the left-hand limb of a letter V, whereas the course of the anterior portion of a right helix will parallel the right-hand limb of the letter V. This will be true no matter in which position the spiral is held. Just as a left-hand screw will be a left-hand screw whether it is driven into a ceiling or a floor, the twist of an umbilical cord is the same whether it is oriented with respect to the fetal or the placental end or whether it is regarded as an independent object.[1]

Placenta records of all infants delivered at our institution in 1985 were reviewed to determine the incidence of left, right, and absent twist of the umbilical cord and the incidence of associated placental abnormalities including immaturity, infarcts, abruptions, meconium staining, circumvallation, succenturiate lobe, congestion, single umbilical artery, cysts, inflammation, and velamentous insertion. There were 2801 live-born singleton placentas with adequate documentation of

Table I. Direction of the umbilical cord twist

	n	Left/right (No.)	Left		Right		Absent	
			No.	%	No.	%	No.	%
Live-born singletons	2801	7.8	2334	83	333	12	134	5
SUA	54	2.8*	34	63	12	22	8	15†
SUA with problems	18	1.1†	8	44	7	39	3	17‡
SUA without problems	36	5.2	26	72	5	14	5	14‡
All twins	368	5.7	290	79	51	14	27	7‡
Monochorionic twins	142	7.8	117	82	15	11	10	7
Dichorionic twins	226	4.8‡	173	77	36	16	17	8
Intrauterine deaths	71	8.7	52	73	6	8	13	18†

SUA, single umbilical artery.

The left/right ratio for SUA with problems is significantly different from that for SUA without problems ($p < 0.05$), but the absent twist incidence is not significantly different. The left/right ratio and absent twist incidence are not significantly different for monochorionic and dichorionic twins.

*$p < 0.01$.

†$p < 0.001$.

‡$p < 0.05$.

Table II. Placental abnormalities

	Left twist (%) (n = 2334)	Right twist (%) (n = 333)	Absent twist (%) (n = 134)
Normal	46.7	43.8	41.0
Immaturity	5.5	5.7	7.5
Infarct	20.6	21.9	22.4
Abruption	4.0	4.8	5.2
Meconium staining	14.6	15.6	19.4
Circumvallation	3.6	4.2	3.0
Succenturiate lobe	8.6	7.5	5.2
Congestion*	1.6	2.1	4.5
Single umbilical artery	0.4	0.6	0.7
Cyst	0.3	0.3	0.0
Inflammation†	2.1	4.8	4.5
Velamentous insertion	1.1	1.2	0.0

*$p < 0.05$.

†$p < 0.001$.

cord twist. In addition, we evaluated 71 placentas associated with singleton fetal deaths collected during a 2-year period, 184 placentas associated with live-born and stillborn twins collected during a 4-year period, and 57 placentas associated with live-borns and fetal deaths with single umbilical artery collected during a 4-year period.

Two hundred consecutive placentas and cords with right twists and 200 consecutive placentas and cords with left twists were ascertained from the placenta records of live-born singleton infants born in 1982. These cases were compiled, and the cord twists were concealed. Families were initially interviewed by telephone. A handedness questionnaire[13] was completed for the child and both parents. A medical and developmental history and a complete family history for left-handedness were obtained. Each child was then examined according to an examination protocol designed to evaluate hand preference and performance. The protocol included coloring, figure drawing, eating, clapping, placing marbles into a small hole, and placing key-shaped pegs into a grooved pegboard (Lafayette Instrument Company, Lafayette, Indiana). For some tasks the child was allowed to choose which hand he preferred to use, whereas for other tasks the child was asked to perform the taks with each hand in succession. The interviews and examinations were administered and scored by a single examiner (R. V. L.). The family and examiner were blinded with respect to the umbilical cord twist of the child. The results of the handedness evaluation were confirmed during a follow-up interview 6 to 12 months after the actual examination. Forty-five children, aged 3 to 4 years, were enrolled and completely evaluated with respect to hand preference and performance.

Data were tested for independence by χ^2 analysis. Probabilities of <5% ($p < 0.05$) are reported as statistically significant. To evaluate the effect of fetal death,

Table III. Umbilical cord twist in 184 twin pairs

	All twins (n = 184)		*Monochorionic twins (n = 71)*		*Dichorionic twins (n = 113)*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Left/left	120	65	48	68	72	64
Right/right	7	4	0	0	7	6
Right/left	35	19	14	20	21	19
Left/absent, right/absent, absent/absent	22	12*	9	13*	13	11*

*$p < 0.001$.

Table IV. Umbilical cord twist and handedness

	Left twist (n = 23)		*Right twist (n = 22)*		*Total (n = 45)*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Right-handed subjects	17	74	19	86	36	80
Non-right-handed subjects	6	26	3	14	9	20
Left-handed subjects	5		1		6	
Mixed-handed subjects	1		2		3	

p = NS.

Table V. Left/right ratios

Study	*Total cases (No.)*	*Left/right ratio*	*Mixed or absent twist (%)*
Hunter[1]	32	7.0	0
Neugebauer[1]	160	2.9	4
Read[1]	54	5.2	8
Hyrtl[1]	120	5.7	16
Shordania[1]	199	1.1	0
Edmonds[1]	100	6.8	6
Malpas and Symonds[4]	707	3.9	8
Chaurasia and Agarwal[3]	528	4.3	20
Present study	2801	7.0	5

twinning, and single umbilical artery on the left/right ratio and incidence of absent twist, we used the 2801 live-born singletons as a control group.

Results

Among live-born singletons, the left twist outnumbered the right twist by 7 to 1 (Table I). Five percent of cords had no twist. The incidence of placental abnormalities was not significantly different between the left, right, and absent twist groups except for congestion and inflammation (Table II).

Among 54 singleton cords with a single umbilical artery, the left-to-right twist ratio was 2.8 to 1 (Table I). The incidence of absent twist (15%) was significantly higher than that for cords with two umbilical arteries ($p < 0.001$). Thirty-seven percent of infants with single umbilical artery were documented to have an associated major structural abnormality, multiple malformation syndrome, or fetal death ("single umbilical artery with problems"; Table I). Although the incidence of absent twist among infants with single umbilical artery with and without problems was significantly higher compared with control subjects, only the infants with problems showed a significant decrease in the left-to-right ratio ($p < 0.001$). There were three sets of twins in which one twin had single umbilical artery. In each set, both cords had left twists.

In only three cases of single umbilical artery were we able to document the side of the absent artery at autopsy. In each of the three cases, the right umbilical artery was either missing or extremely hypoplastic. Two subjects with completely absent right artery had right twists. The one patient with extremely hypoplastic right artery had a left twist.

Among 184 twin pairs (Table III), 120 (65%) were concordant for left twist, only seven (4%) were concordant for right twist, and 35 (19%) showed one left and one right twist. The remainder (22 twin pairs, 12%) showed no twist in one or both twins ($p < 0.001$). When each member of a twin pair was treated as an individual, left twists outnumbered right twists by 7.8 to 1 for monochorionic twins and by 4.8 to 1 for dichorionic twins. The number of individual twins with no twist was 7% for monochorionic and 8% for dichorionic twins (Table I).

Among 71 cords associated with singleton fetal deaths (Table I), 73% had left twists, 8% had a right twist, and 18% had no twist ($p < 0.001$ for absent twist).

Of the 45 children evaluated for handedness (Table IV), 23 were from the left twist group and 22 were from the right twist group. There were no major medical or developmental problems with either group. There were no significant differences in frequency of first-, second-, or third-degree relatives who were left-handed. The number of non-right-handed children (20%) is consistent with previously reported estimates for the general population.[11] There were only four mothers who were left-handed, indicating that the twist is independent of the handedness of the mother. Furthermore, the prevalence of right- versus non-right-handed mothers was not significantly different between the two groups. Among the 23 children in the left twist group, 17 (74%) were right-handed and six (26%) were non-right-handed (five left-handed and one mixed-handed). Among the 22 children in the right twist group, 19 (86%) were right-handed and three (14%) were non-right-handed (one left-handed and two mixed-handed).

Comment

A variety of theories have been proposed to explain the umbilical cord twist, most of which fall into two general groups: those that interpret the twist as inherent to the cord itself and those that explain the twist as the effect of active or passive rotation of the fetus.[1] The data set forth in this report suggest that both factors are relevant: that the impetus for cord twisting is inherent to the umbilical cord itself and that the normal twist can be impeded by any factor that restricts normal fetal movement. Supporting evidence documented in this study as well as from the literature is as follows.

Malpas and Symonds[4] described an experimental model that provided evidence that the umbilical cord twist is a property intrinsic to the cord itself. Hollow tubes whose walls were constructed from fibers with inbuilt torsion were shown under hydrostatic pressure to exhibit the typical helical shape of the cord. The direction of the twist of the tube was contrary to the direction of the twist of the fibers constituting its walls. They postulated that the helical configuration protected blood flow because it enabled the cord to withstand torsion without snarling.

Several authors have previously proposed theories that stress the importance of asymmetry in size of the umbilical arteries. Grosser explained the twist as the result of unequal growth of the umbilical arteries, whereas Simpson held that a rotational torque resulting from differential blood flow between the left and right umbilical arteries could produce the twist in the umbilical cord.[1] The fact that the right umbilical artery is usually larger than the left umbilical artery would explain why the left twist is more common than the right twist. In fact, among live-born singletons in this study the left twist outnumbered the right twist by 7 to 1. Previous authors have reported left/right ratios ranging from 1.1 to 7.0[1, 3, 4]; however, the largest study to date included only 707 umbilical cords compared with the 2801 cords in the present study (Table V).

Among 54 cords with a single umbilical artery in this study, the incidence of absent twist was significantly increased among infants with single umbilical artery. If the origin of the twist is dependent on the torque resulting from differential blood flow between the two umbilical arteries, then in the presence of a single umbilical artery the incidence of absent twist would be expected to increase. Two further features noted among cords with single umbilical artery are relevant with respect to the theory that the impetus for the cord twist comes from within the cord itself and is dependent on the dynamic forces arising from differential blood flow: (1) the left-to-right twist ratio decreased to 2.8, largely as a result of the random distribution of left and right twists in the infants with single umbilical artery with problems (left-to-right ratio of 1.1) and (2) in the two cases of completely absent right umbilical artery, the cord twist was to the right.

The cause and time of onset of the single umbilical artery probably are different for infants with isolated single umbilical artery as compared with infants with malformation syndromes in which single umbilical artery is one feature. In the latter group the missing umbilical artery most likely represents a primary defect in embryologic development and is present before the initiation of the cord twist and therefore can affect its direction. Assuming a random loss of either the right or left umbilical artery, the direction of the cord twist would be expected to relate to the remaining or dominant artery. In the two cases of completely absent right umbilical artery documented in this study, as one would expect, there was a right cord twist conforming to the rotational torque imposed on the cord by blood flowing through the remaining left umbilical artery. The single umbilical artery in otherwise normal infants most likely develops after 42 days' gestation secondary to atrophy

of one umbilical artery, is not present until after the cord twist is established, and thus has no influence on its direction.

Previous reports have documented short umbilical cords that lack a twist in both humans and animals in which fetal activity has been restricted, suggesting that the cord twist also can be affected by active or passive rotation of the fetus.[5-10]

A particularly popular theory referred to here as the "bathtub vortex theory" has suggested that the fetus in utero rotates in a counterclockwise direction in the northern hemisphere and in a clockwise direction in the southern hemisphere similar to the direction of rotation of a hurricane or water draining out of a bathtub.[14] However, of 36 placentas examined in Australia, 24 had a left cord twist, eight had a right twist, and four were indeterminate, tending to discount the possible influence of the bathtub vortex on the cord twist phenomenon (Short RV, Brennecke S, personal communication, 1986).

Careful evaluation of hand preference and performance of a group of 3- and 4-year-old children ascertained by their cord twist indicates that the handedness of neither the child nor the mother correlates with the direction of the twist of the umbilical cord. Although these data discount the initial hypothesis set forth in this article that the predominance of left twists is the result of more forceful paddling with the right arm of a fetus who has already established handedness, it does not negate the theory set forth by a number of investigators that the cord twist is the result of either active or passive rotation of the fetus.[1] Documentation in this study of an increased incidence of absent twists among twins gives credence to the theory that fetal rotation is necessary for the normal umbilical cord twist to occur. By virtue of intrauterine crowding, twins have an increased incidence of constraint-related defects including short umbilical cords.[15] It is therefore not surprising that rotation of the fetus to either the left or right would occur less frequently in twins than in singletons. Furthermore, documentation of an increased incidence of absent twists among spontaneous abortuses and stillbirths suggests that decreased fetal activity as a result of fetal defects can also adversely affect cord twisting. Thus, decreased fetal activity as a result of intrinsic (fetal) and extrinsic (intrauterine constraint) causes can affect the cord twist phenomenon.

Although the data set forth in this article indicate that the origin of the umbilical cord twist reflects the rotational forces within the cord itself caused by differential blood flow between the two umbilical arteries, these data also indicate that decreased fetal activity plays a role in impeding normal cord twisting. That the umbilical cord most frequently rotates to the left as a result of asymmetric blood flow between the right and left umbilical arteries is further indication of the developing organism's propensity for asymmetric development.[11] Just as a short umbilical cord has been associated with an increased incidence of neurologic problems and developmental delay, absence of a cord twist may be associated with adverse developmental prognosis.

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Indicators of perinatal asphyxia

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Creatinine phosphokinase and its isoenzymes (myocardial-specific MB and brain-specific BB) are elevated in the presence of specific tissue injury. The value of this serum marker as an objective indicator of perinatal asphyxia was studied. Forty-nine patients with gestational ages ranging from 36 to 42 weeks were prospectively studied. Patients who by interpretation of the fetal heart rate tracing alone were diagnosed as having fetal distress demonstrated significantly lower pH ($p = 0.001$) and base excess ($p < 0.0001$) in umbilical venous blood. Umbilical venous cord MB ($p < 0.05$) and BB ($p < 0.01$) were increased in this group. Abnormal fetal heart rate patterns correlate well with acid-base abnormalities and elevated creatinine phosphokinase values. These tests may demonstrate more efficient and improved indicators of tissue injury and damage from perinatal asphyxia than clinical observation alone. (Am J Obstet Gynecol 1987;157:839-43.)

Key words: Asphyxia, creatinine phosphokinase, acid-base balance

Intrapartum asphyxia has long been recognized as a major contributor to neonatal morbidity, mortality, and long-term sequelae.[1] It has been singled out by some authors as the leading cause of neurologic morbidity in term infants as well as significantly influencing the morbidity in the preterm neonate.[2] Despite the magnitude of its importance, the accurate and objective diagnosis of this disorder remains elusive.

Apgar scoring,[3] which was developed to quantitate the initial evaluation of the newborn infant and direct medical attention to infants in need of immediate resuscitation, has been the most widely used index of asphyxial assessment at birth. However, Apgar scores are not specific for hypoxia, and they do not appear to closely correlate with intrapartum indicators of asphyxia.[4]

Acid-base analysis of umbilical cord blood provides objective information on the acute and chronic respiratory and metabolic condition of the fetus at birth. Although it appears that this analysis may provide specific criteria for evaluation of the condition of the fetus at birth, its validity has been tested only against Apgar scoring.[4, 5] Similarly, its value as a true predictor of the condition of the fetus is best judged when maternal acid-base analysis is normal, since maternal respiratory and metabolic derangements may falsely increase or decrease the fetal pH value.

Therefore there is no ideal measure of perinatal asphyxia in clinical practice. Apgar score, fetal heart rate monitoring, and umbilical cord blood gas analysis have been shown to not correlate well with asphyxia.[1-6] More specific and objective criteria need to be developed and tested.

Creatinine phosphokinase and its isoenzymes, BB or brain-specific fraction (CPK_1), MB or myocardial-specific fraction (CPK_2), and the muscle-specific fraction MM (CPK_3) are known to elevate in response to muscle trauma, myopathy, severe exertion, or muscle hypoxia. Therefore the use of this marker as a detector of tissue-specific damage in response to intrapartum fetal distress is quite intriguing.

Material and methods

Forty-nine patients who came to the University of Maryland Hospital between Oct. 1, 1984 and May 31, 1985 were studied. Eligibility was based on a gestational age of 36 weeks or greater, electronic fetal heart rate monitoring for a minimum of 30 minutes, and written informed consent. No other factors that would potentially affect the values of our creatinine phosphokinase levels such as Duchenne-Landouzy dystrophy, tetany, fenoterol, hypothyroidism, or extreme trauma were present for study inclusion. At the time of delivery, the umbilical cord was doubly clamped and divided. Samples of umbilical arterial and venous blood were drawn into heparinized syringes and analyzed by a Corning 170 blood gas analyzer with base excess calculated by the Siggard-Anderson[7] nomogram. Umbilical arterial values were used for statistical analysis of blood gas results.

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Table I. Categoric definitions of patients

Group	*Interpretation*	*Definition*
I (n = 20)	Normal	No fetal heart rate abnormalities
II (n = 9)	Mild distress	Fetal heart rate change noted; no intervention recommended
III (n = 20)	Moderate to severe	Fetal heart rate change severe enough to mandate treatment—scalp pH or delivery

Umbilical venous cord blood was simultaneously obtained at the time of delivery from the same doubly clamped cord specimen for evaluation of creatinine phosphokinase isoenzymes. A second venous serum sample was drawn from the neonate at 30 hours of age (±6 hours) for isoenzyme activity of creatinine phosphokinase. Immediately after the specimens were drawn, the samples were transported on ice to the laboratory where analysis was performed with the Beckman Paragon creatinine kinase isoenzyme electrophoresis kit (Beckman Instruments, Inc., Palo Alto, Calif.). The principles of this procedure are based on creatinine phosphokinase catalyzing the reversible transer of a phosphate group from creatinine phosphate to adenosine diphosphate forming adenosine triphosphate. The rate of adenosine triphosphate formation is measured through the use of the two coupled reactions catalyzed by hexokinase and glucose 6-phosphate dehydrogenase. The rate of reduction of nicotinamide adenine dinucleotide to nicotinamide adenine dehydrogenase is followed by measuring the increase in absorbance at 340 nm. The rate of change in absorbance is directly related to the activity of creatinine phosphokinase in the specimen.[8] Electrophoretic separation of the isoenzymes of creatinine phosphokinase may then be quantitatively analyzed when subjected to densitometric scan, and the relative amounts of the different isoenzymes are calculated as percentages and then finally recorded as international units per liter of creatinine phosphokinase activity.

Analysis of fetal heart rate tracings was performed by a perinatologist who was unaware of the patients' identity and eventual outcome. The categoric division of patients by this method is demonstrated in Table I. Neonatal evaluation was made up of Apgar scoring at 1 and 5 minutes by the obstetric and pediatric staff and confirmation of gestational age was by Dubowitz examination. Results were analyzed with the Spearman rank correlation coefficient and Mann-Whitney U test where appropriate. Nonparametric techniques were used despite the probability of a decrease in power since our data did not appear to be distributed normally.

Results

Table II summarizes the correlation between cord blood gas analysis and creatinine phosphokinase isoenzymes at the two sampling points. As predicted, cord pH was correlated with base excess. The myocardial-specific isoenzyme of creatinine phosphokinase (CPK_2) at birth was significantly correlated with the base excess of the umbilical cord ($r = 0.36$, $p < 0.005$). At 30 hours, both the heart-specific fraction (CPK_2) and the brain-specific isoenzyme of creatinine phosphokinase (CPK_1) were significantly correlated with the degrees of base excess present in umbilical cord blood samples. Although there was not a significant correlation between cord pH and isoenzyme fractions of creatinine phosphokinase at either time point, there tended to be a higher correlation between isoenzyme levels and base excess levels from the umbilical cord than with cord pH at both sampling times.

Division of patients on the basis of interpretation of fetal heart rate (Table III) was then performed, and the differences between groups were analyzed. There were significant differences between infants with normal fetal heart rate tracings as opposed to those demonstrating electronic evidence of moderate to severe fetal distress in umbilical cord pH ($p = 0.001$) and base deficit ($p < 0.0001$). The BB (CPK_1) isoenzyme level in group III (moderate severe distress) infants was significantly greater ($p < 0.01$) at birth than the corresponding level in infants with normal fetal heart rate tracings (group I). Although the second sample taken at 30 hours of age demonstrated that the BB (CPK_1) isoenzyme level of infants with moderate to severe fetal heart rate abnormalities was higher than in those with normal heart rate tracings, it failed to reach statistical significance. Examination of the MB (CPK_2) fraction revealed similar findings, with the isoenzyme at birth being significantly greater ($p < 0.05$) in group III infants while those samples drawn at 30 hours of age, although seemingly higher, failed to demonstrate statistical significance in comparison with the normal group.

The population was divided into two groups: group A, cord pH of ≤7.25 versus group B, cord pH of >7.25. This value was chosen, since it represents a period of preacidosis whereby plans for potential intervention in the birth process should be begun.[9] This separation demonstrated that the MB isoenzyme of the umbilical cord was significantly elevated ($p < 0.05$) in group A versus group B (Table IV). The MB fraction from the neonate at 30 hours of age, however, was not found to be significantly different in the two groups despite the increased acidosis present at birth in group A. The BB isoenzyme of creatinine phosphokinase was

Table II. Entire study population

	pH	*Base excess*	*CPK_1 (cord)*	*CPK_1 (30 ± 6 hr)*	*CPK_2 (cord)*	*CPK_2 (30 ± 6 hr)*
pH	—					
Base excess	0.46‡	—				
CPK_1 (BB) (cord)	0.07	0.19	—			
CPK_1 (BB) (30 ± 6 hr)	0.22	0.40†	0.08	—		
CPK_2 (MB) (cord)	0.06	0.36†	0.19	0.37†	—	
CPK_2 (MB) (30 ± 6 hr)	0.14	0.32*	0.06	0.56‡	0.39†	—

*$p < 0.01$.
†$p < 0.005$.
‡$p < 0.0001$.
Significance testing was with the Spearman rank correlation coefficient.

Table III. Division and analysis of patients based on interpretation of fetal heart rate

	Group I (no distress)	*Group II (moderate to severe distress)*	*p Value**
pH	7.33	7.23	$p = 0.001$
Base excess	−4.0	−7.9	$p < 0.0001$
CPK_1-BB (cord)	21 IU/L	31 IU/L	$p < 0.01$
CPK_1-BB (30 ± 6 hr)	21 IU/L	25 IU/L	NS
CPK_2-MB (cord)	16 IU/L	28 IU/L	$p < 0.05$
CPK_2-MB (30 ± 6 hr)	28 IU/L	34 IU/L	NS

*Significance testing was by Mann-Whitney U test.
All values reported are median result.

Table IV. Comparison of groups A and B

	Group A ($pH \leq 7.25$)	*Group B ($pH > 7.25$)*	*p Value**
Base excess	−9	−5	$p = 0.0001$
CPK_1-BB (cord)	28 IU/L	16 IU/L	NS
CPK_1-BB (30 ± 6 hr)	30 IU/L	31 IU/L	NS
CPK_2-MB (cord)	30 IU/L	22 IU/L	$p < 0.05$
CPK_2-MB (30 ± 6 hr)	28 IU/L	21 IU/L	NS ($p = 0.06$)

*Significance testing was by Mann-Whitney U test.
All values reported are median result.

unable to differentiate the groups at either sampling time periods. Identical results were obtained when the division was based on base excess (group A = base excess ≤ -10) (group B = base excess > -10).

Comment

The present study demonstrates strong support that creatinine phosphokinase and its isoenzymes are elevated when there is fetal distress as judged by electronic monitoring of fetal heart rate. These increases are presumed to occur as a result of ischemia-induced leakage of enzymes from damaged cells in tissue in which the isoenzymes are concentrated. In conjunction with this rise in enzyme activity is a lowering of cord pH values and an increase in base deficit, which probably indicate that hypoxic damage has occurred.

Examination of the population as a whole demonstrated a significant correlation between the degree of base excess and creatinine phosphokinase activity (Table II). This same association, however, is not found in conjunction with pH values. Hypoxia and/or asphyxia result in diminution of oxygen tension and an increase in carbon dioxide tension levels. In time this leads to anaerobic metabolism and lactic acidosis. Acid-base status may change rapidly in response to the changes in P_{CO_2} values, but base excess reflects a damped or conservative picture of the magnitude of the metabolic changes from normal, changing only after prolonged hypercarbia. In a similar manner, the myocardial-specific isoenzyme (CPK_2), with its longer half-life (peak rise 24 hours after injury), would appear more pivotal as an assessment of asphyxial insults than

its shorter-lived counterpart, the brain-specific isoenzyme (CPK_1) (peak rise 6 hours after injury). This is in fact our observation. Therefore explanation of the correlation of CPK_1 at 30 ± 6 hours of age with base excess leads to the hypothesis that this enzyme increase is not a primary response to the birth process but perhaps a secondary response to some degree of myocardial injury that has occurred during or after labor and delivery.

In 1966 Rudolph and Gross[10] first reported creatinine phosphokinase values at 24 hours of age in infants delivered by emergency cesarean section, which followed prolonged labors and oxytocin infusion and after delivery from breech presentations. They suggested that muscle trauma was the likely cause for these increases. In 1978 Becker and Menzel,[11] who examined the value of the brain-specific creatinine kinase enzyme in the serum of newborn infants with perinatal damage, demonstrated a pattern of neuropathologic symptoms, an increase in total activity of serum creatinine kinase, and an increase in the brain-specific isoenzyme when there is central nervous system damage and an unfavorable prognosis. Warburton et al.[12] suggested that elevated levels of creatinine phosphokinase reflected perinatal asphyxia. Their data demonstrated a correlation between acidosis, 1-minute Apgar scores, and isoenzyme levels of creatinine phosphokinase in the neonate infant. These data, however, are only based on neonatal data collection without reference to intrapartum events or differentiation between the acute and chronic respiratory and metabolic condition of the neonate.

Subsequent research has been unable to clarify clearly the relationship between abnormal fetal heart rate patterns, asphyxia, and isoenzyme elevations of creatinine phosphokinase in the newborn. In 1985 Feldman et al.[13] demonstrated a direct association between ominous fetal heart rate patterns and cord BB, although there was no significant elevation of the creatinine phosphokinase myocardial fraction. In contrast, Woolley et al.[14] reported increases in MB activity at 36 ± 6 hours of age, although not at birth in neonates with abnormal fetal heart rate tracings. Neither study investigated the relationship between the metabolic status of the infant at birth and creatinine phosphokinase levels.

The diagnosis of fetal distress by electronic fetal heart rate tracings is known to be fraught with many false-positive interpretations.[15] The classification of these recordings by the simplified system we used is less than ideal. Scoring indexes have been developed and may be more specific as diagnostic tools.[16] The timing of when changes in the fetal heart rate pattern began and their duration may have aided the mapping of enzyme rises. Stress precedes distress, and it is known that a considerable time delay occurs before biochemical parameters fall outside the physiologic normal range. However, our data, as well as previous investigations by others, demonstrate that tissue injury appears to occur when fetal heart rate patterns are abnormal. The paucity of neonatal follow-up in our study prohibits the accurate assessment of the clinical significance of this finding. These deficiencies are currently being corrected.

The value of creatinine phosphokinase and its isoenzymes as a way to identify the severity of fetal distress at this time is limited. Wide fluctuations in the isoenzyme results obtained would lead to considerable confusion in the diagnosis of distress with the means currently available. The potential validity of creatinine phosphokinase isoenzymes as markers of fetal/neonatal asphyxia must not be overlooked. The significant correlation that CPK_1 and CPK_2 demonstrate with respect to the acid-base status of umbilical cord sera and their concomitant relationship with abnormalities in fetal heart rate lend support to the possibility that these markers may play a role as predictors of fetal hypoxia. Equally intriguing is the failure of these parameters to correlate with the present gold standard to many in clinical practice today, Apgar scores. Results of ongoing analyses of changes in fetal heart rate responses to labor with respect to time in relation to cord blood determination of creatinine phosphokinase may shed light on whether isoenzyme analysis will succeed other techniques as markers of perinatal hypoxia.

In addition, it would seem prudent to attempt to establish specific and objective criteria for the evaluation of fetal outcome other than Apgar scores. One must remember that the identification of asphyxia is based on hypoxia, hypercarbia, and acidosis. In turn perinatal asphyxia may then lead to tissue damage of the heart and brain. The role that creatinine phosphokinase isoenzyme levels and umbilical cord acid-base studies may play needs delineation. Prospective evaluation of these parameters with respect to specific fetal heart rate changes, their timing and duration, and fetal outcome through more vigilant study of the neonatal course, neurologic performance, and myocardial status of the newborn is needed. This knowledge will hopefully lead to more efficient indicators of tissue injury and the presence or absence of asphyxial changes. Perhaps armed with this knowledge, we will be better prepared to monitor improvements in antenatal and intrapartum care.

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Umbilical cord blood pH and Apgar scores as an index of neonatal health

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In an effort to determine the clinical usefulness of Apgar scoring and cord pH in neonatal management, specimens of umbilical cord blood were obtained after 257 of 329 consecutive deliveries at a community-based hospital. A notable number of babies who were born in a vigorous state were in fact acidotic (umbilical arterial pH >1 SD below mean). Seventy-two percent of acidotic babies had an Apgar score >7 at 1 minute, and 92% had an Apgar score >7 at 5 minutes. Correlation coefficients of Apgar scores at 1 and 5 minutes and arterial pH with the health status of newborn infants were poor. A χ^2 analysis of arterial pH and Apgar scores at 1 and 5 minutes indicated that a larger number of sick babies had an Apgar score <7 and acidosis than expected by pure chance. However, the sensitivity values of the Apgar score at 1 minute (0.48) and 5 minutes (0.24) and the arterial pH (0.40) for predicting sick children limit the clinical usefulness of these tests. While technically feasible in a community hospital, routine cord pH measurements add little to neonatal evaluation and management. (AM J OBSTET GYNECOL 1987;157:843-8.)

Key words: Acidosis, arterial pH, Apgar score

Rapid neonatal assessment of intrapartum and delivery acidosis and asphyxia as an index for various neonatal risks was the intent of Virginia Apgar when she introduced her system in 1953. This was also the purpose of her research in the ensuing years.[1] However, the application of this system is limited today because of improved newborn resuscitation and neonatal care, which have resulted in improved Apgar scores at 5 minutes.

Many contend that neurological abnormalities of the infant are a result of oxygen deprivation before delivery and are infrequently compounded by asphyxia of the newborn infant itself. It is difficult to determine whether poor Apgar scores are the result of oxygen deprivation before delivery or expressions of already abnormal babies. Therefore the long-term predictive value of the Apgar system is questionable. In fact, large collaborative studies completed in the late 1960s[2] in-

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Table I. Correlation matrix for major variables

		Apgar score		
	Health	*1 min*	*5 min*	*Arterial pH*
Health	1.0000 329			
Apgar score				
1 min	0.5620 329	1.0000 329		
5 min	0.4629 329	0.7364 329	1.0000 329	
Arterial pH	0.2454 257	0.3454 257	0.1885 257	1.0000 257
Venous pH	0.2230 273	0.3087 273	0.1919 273	0.7936 273

The top number is the correlation coefficient. The bottom number is the total number of cases.

dicated that >80% of children with 5-minute Apgar scores <7 did not have abnormal neurological findings at 1 year of age. More recent data indicate that Apgar scores are only minimally related to neurological outcome in either the short or long term.[3-9]

The Apgar system often seems to be subjective in its applications, and it has shortcomings. Numbers are often assigned in retrospect. Scores are often inflated secondary to provider inexperience, ego, or fear of criticism by management. The timing of Apgar score assignment is not always coincidental with 1 and 5 minutes after birth. Umbilical artery data at delivery should correlate more closely with the metabolic status of the fetus just before birth than subjective Apgar scoring.

Sykes et al.[10] evaluated large numbers of umbilical arterial cord blood samples as an objective assessment of intrapartum and delivery acidosis. Of their babies with a 1-minute Apgar score <7, only 21% were acidotic, and only 19% of those with an Apgar score at 5 minutes <7 were acidotic. Of the babies with arterial cord blood pH <1 SD below the mean, 73% had an Apgar score at 1 minute >7 and 86% had an Apgar score at 5 minutes >7. These results indicate that Apgar scores are poor predictive indicators of fetal acidosis. A recent study by Silverman et al.[11] of 1032 neonates also indicated that, except in the case of severe blood gas abnormalities, umbilical artery metabolic status is not closely related to Apgar score. All blood gas parameters—including pH—failed to predict which infants were depressed according to the Apgar score.

Our prospective study was undertaken in an attempt to correlate Apgar scoring with newborn acid-base status in a smaller and less high-risk population hospital setting than that used in previous research.[10, 11] Most important, the early neonatal course was followed up to determine the clinical usefulness of Apgar scoring, cord blood pH testing, and the combination of the two modalities in neonatal management.

Material and methods

Venous and arterial cord blood samples were drawn from a segment of triple-clamped cord. The cord was clamped immediately after delivery, and blood sampling was completed as soon after delivery as possible. Lievaart and deJong[12] have shown that cord clamping is necessary to keep umbilical cord blood pH, carbon dioxide partial pressure, and base deficit values close to time zero results. Samples were obtained in consecutive deliveries without regard to maternal or fetal condition, course of labor and delivery, or establishment of respirations.

Each specimen was drawn into a standard arterial blood gas syringe that had been preheparinized with 1 ml of 1000 U/ml of heparin solution. Arterial and venous punctures were completed with a 20-gauge needle.[13] Residual air was ejected, the syringe was capped with a provided plastic cover, the specimen was placed on ice, and blood gas determinations were completed as soon as possible (preferably within 60 minutes). Strickland et al.[14] have shown that there is minimal change in arterial blood pH values if analysis is completed in <120 minutes. Other researchers have shown that icing cord blood samples does not change the rate of arterial pH decrease.[15] The cord blood gas analysis was completed on an Instrumentation Laboratories Model 1303 analyzer with documented accuracy and daily calibration.

Data collected for each patient included hospital number, date and time of delivery, Dubowitz (gestational age) score, Apgar scores at 1 and 5 minutes, birth weight in grams, race, arterial and venous cord blood gas study results, and time of blood gas analysis completion. The neonatal course of each baby was also recorded. Nursery personnel were not informed of any blood gas study results, in an effort to limit bias in their management. Consistency of diagnosis and management was assumed by a single neonatologist attending physician during the study period. Acidosis was operationally defined as arterial pH at least 1 SD below the mean. Abnormal Apgar scores were defined as <7 at 1 and 5 minutes. Newborn infants placed in the neonatal intensive care unit for observation and treatment because of clinical illness (excluding hypoglycemia and hyperbilirubinemia) were classified as "sick." These babies were those with meconium aspiration (two), subarachnoid hemorrhage (one), pneumothorax (one), polycythemia (one), respiratory distress syndrome (two), transient tachypnea (three), and radiographic evidence of pulmonary disease requiring oxygen support (seven) and those receiving antibiotics for signs of sepsis (eight). A relationship between these illnesses and prenatal and perinatal events has been widely accepted.

Table II. Comparison of the Apgar score at 1 minute and arterial pH

	Apgar score at 1 min				
	<7		>7		
*Arterial pH**	*n*	*%*	*n*	*%*	*No.*
≥1 SD below mean	11	28	28	72	39
<1 SD below mean	10	5	208	95	218
	21		236		257

$\chi^2 = 21.546$; df = 1; $p < 0.0001$.
*Arterial pH ≥1 SD below mean indicates acidosis.

Table III. Comparison of the Apgar score at 5 minutes and arterial pH

	Apgar score at 5 min				
	<7		>7		
*Arterial pH**	*n*	*%*	*n*	*%*	*No.*
≥1 SD below mean	3	8	36	92	39
<1 SD below mean	3	1	215	99	218
	6		251		257

$\chi^2 = 27.205$; df = 1; $p < 0.00001$.
*Arterial pH ≥ 1 SD below mean indicates acidosis.

Results

Data were analyzed in two stages. First, to rule out selection bias, an in-depth analysis was performed to verify that the cases for which pH data were not obtained were not significantly different from the study population. Second, the major question of this study, the clinical usefulness of Apgar scoring and cord blood pH testing in neonatal management, was addressed.

Specimens of cord blood were drawn after 257 of 329 consecutive deliveries over a 14-week period in a level 2 community-based hospital. Only those cases with arterial and venous control values were included in the study. Of the 257 deliveries for which both arterial and venous data were available, 224 (87%) were vaginal deliveries and 33 (13%) were cesarean deliveries. Blood pH data were not obtained in 72 deliveries (21.9%) because of inadequate sampling (16 cases with venous pH data only), inadequate manpower, or technical difficulties with cesarean deliveries completed in the operating room. The problem with the operating room accounted for a significantly larger number of cesarean deliveries being missed (37.5%) as compared with vaginal deliveries (19.7%). Thirty-four children were labeled as sick with clinical illness and received treatment consisting of oxygen support or antibiotics. Blood pH data were missing for only 21.4% of babies labeled as being well and for 26.5% of babies labeled as sick. This was not statistically significant. In agreement with previous research,[16] a high correlation was noted between arterial pH and venous pH values (r = 0.7936); therefore we elected to use arterial pH values as the basis for our analysis. Because combining other blood gas data (bicarbonate deficit or partial pressures of carbon dioxide or oxygen) with pH has been shown to add only a slight advantage in the prediction of infants with low Apgar scores,[11] and because our data were consistent with this, we report pH values alone for simplicity and practicality.

Mean time from delivery to blood gas analysis was 71 minutes. There was no correlation between the interval from delivery to blood gas analysis and arterial pH.

The race distribution was 64.6% white, 30.7% black, and 4.7% other (Oriental, Hispanic, and mixed). There was no significant difference in the percentage of white (19.8%), black (24.8%), and other (23.5%) babies missed for all deliveries.

Multiple *t* test analyses were completed to determine if there were differences between cases with and cases without arterial pH data. There were no significant differences, suggesting that the two populations are essentially equivalent across the variables critical to this study and that no selection bias existed for those cases without arterial pH gas data (data available on request).

The mean arterial pH of all 257 cases was 7.2171 (SD = 0.0756). The mean arterial pH of sick babies was 7.161 (SD = 0.1127), and that of well babies was 7.2232 (SD = 0.0681). The difference between the

Table IV. Comparison of arterial pH and health status

	Arterial pH				
	≥1 SD below mean		*<1 SD below mean*		
	n	*%*	*n*	*%*	*No.*
Sick	10	40	15	60	25
Well	29	13	203	87	232
	39		218		257

$\chi^2 = 11.209$; df = 1; $p < 0.0008$. Sensitivity = 0.40. Specificity = 0.875.

Table V. Comparison of Apgar score at 1 minute and health status

	Apgar score at 1 min				
	<7		*>7*		
	n	*%*	*n*	*%*	*No.*
Sick	12	48	13	52	25
Well	9	4	223	96	232
	21		236		257

$\chi^2 = 56.114$; df = 1; $p < 0.00001$. Sensitivity = 0.48. Specificity = 0.96.

Table VI. Comparison of Apgar score at 5 minutes and health status

	Apgar score at 5 min				
	<7		*>7*		
	n	*%*	*n*	*%*	*No.*
Sick	6	24	19	76	25
Well	0	0	232	100	232
	6		251		257

$\chi^2 = 46.971$; df = 1; $p < 0.00001$. Sensitivity = 0.24. Specificity = 1.00.

mean pH values of sick and well babies was statistically significant ($t = 4.012$; df = 255, $p < 0.0001$).

Pearson's r product-moment correlation coefficients of the total population of deliveries for health (sick versus well), arterial pH, Apgar score at 1 minute, and Apgar score at 5 minutes were low, the best being the Apgar score at 1 minute (Table I).

Cross-tabulation analysis of Apgar scores at 1 and 5 minutes versus acidosis (arterial pH >1 SD below the mean) is noted in Tables II and III. The χ^2 analysis for the Apgar score at 1 minute ($X^2 = 21.5459$; df = 1) and at 5 minutes ($X^2 = 27.205$, df = 1) was significant.

Only 52.3% of the babies with an Apgar score at 1 minute <7 and 50% of the babies with an Apgar score at 5 minutes <7 were acidotic. Seventy-two percent of the babies with acidosis had an Apgar score ≥7 at 1 minute, and 92% had an Apgar score ≥7 at 5 minutes.

Analysis of arterial pH, Apgar score at 1 minute, and Apgar score at 5 minutes versus health (sick versus well) were completed. The χ^2 values indicate that the number of sick babies who were also empirically ill, as determined by acidosis (Table IV) or Apgar scores at 1 minute <7 (Table V) or 5 minutes <7 (Table VI) were significantly greater than expected by chance. As was anticipated, all six babies with an Apgar score at 5 minutes <7 were sick. However, the sensitivity value of this measurement for predicting sick children was poor (0.24), as 76% of babies of who were sick had an Apgar score at 5 minutes >7 (Table VII). This was probably related to proper newborn resuscitation measures that might have significantly contributed to outcome. The arterial pH and Apgar score at 1 minute <7 sensitivity values for predicting sick children were also poor. The best was the Apgar score at 1 minute <7 (0.48) (Tables IV and V).

Because acidosis and the Apgar scores at 1 and 5 minutes were not as individual measures sensitive

Table VII. Comparison of Apgar score at 1 minute or arterial pH and health status*

	Low Apgar score or low arterial pH	*Normal Apgar score and normal arterial pH*	*No.*
Sick	15 (60%)	10 (40%)	25
Well	34 (15%)	198 (85%)	232
	49	208	257

$\chi^2 = 27.205$; df = 1; $p < 0.00001$. Sensitivity = 0.60. Specificity = 0.85.
*Apgar score at 1 minute <7 or arterial pH ≥1 SD below the mean.

Table VIII. Comparison of Apgar score at 1 minute or arterial pH and health status*

	Low Apgar score and low arterial pH		*Normal Apgar score or normal arterial pH*		
	n	%	*n*	%	*No.*
Sick	7	28	18	72	25
Well	4	2	228	98	232
	11		246		257

$\chi^2 = 31.887$; df = 1; $p < 0.00001$. Sensitivity = 0.28. Specificity = 0.983.
*Apgar score at 1 minute <7 and arterial pH ≥1 SD below mean.

indicators of health status, composite measures of arterial pH and Apgar scores versus health (sick versus well) were developed (Tables VII and VIII). Again, the χ^2 values are statistically significant; however, even the combination of acidosis or Apgar score at 1 minute <7 produced a sensitivity level of only 0.60.

Comment

It is generally accepted that severe metabolic acidosis is related to highest fetal risk. Apgar scores may not be very good estimates of this risk and are subjective. Umbilical pH determinations may better serve this purpose.

We have shown that accurate cord blood gas analysis can be accomplished in an average-size community hospital (250 beds) with the use of routine blood gas sampling and analysis equipment. An in-depth analysis of population equivalence between cases with and without arterial pH data identified no significant differences between the two. This indicated that cases missed because of technical problems were due to chance, and that selection bias was not present. This population is different than those previously studied because most patients had relatively uncomplicated conditions. There were few preterm infants; most were term and appropriate for gestational age.

In an effort to be consistent with Sykes et al.,[10] we considered an arterial pH >1 SD below the mean (pH <7.14) to be indicative of fetal acidosis. We are in agreement that a high proportion of vigorous babies can be born with severe acidosis (Tables III and V). Also, the correlations between pH, Apgar score at 1 minute, and Apgar score at 5 minutes were poor (Table II). The real dilemma however, is how these and other results[7-11] applies to clinical practice. In our study the usefulness of cord arterial pH and Apgar score values in predicting which babies would be clinically ill during their neonatal hospital course was examined closely. Although the difference between mean arterial pH values for the babies who were sick versus those who were well is statistically significant, the correlations of arterial pH, Apgar score at 1 minute, and Apgar score at 5 minutes to health status (sick versus well) are certainly not impressive. In fact, Apgar scores (especially the Apgar score at 1 minute) correlated better than blood pH values with health status (Table I).

Cross-tabulation analyses of arterial pH, Apgar score at 1 minute, and Apgar score at 5 minutes versus sick and well babies yielded statistically significant differences between the sick and well subpopulations. However, in light of very poor sensitivity levels, these statistical differences may not be useful for routine clinical decisionmaking. In fact, 60% of sick babies were predicted to be well by arterial pH criteria and 52% by Apgar score at 1 minute criteria (Tables IV and V). To obtain a clinically useful test, two composites of Apgar score at 1 minute and/or arterial pH versus health (sick versus well) were tabulated (Tables VII and VIII). The best composite, Apgar score at 1 minute or arterial pH, was only 60% sensitive for sick babies; therefore 40% of sick babies were predicted to be well.

Because the study sample was a low-risk obstetric population from a community-type hospital, more stringent criteria such as a pH 2 SD below the mean (≤7.1) or Apgar scores at 10 or 20 minutes might have been more predictive, as suggested by others.[4,5] On the

other hand, our data support a growing body of information that intrapartum events are less predictive than have been previously believed.[8, 9]

Umbilical cord blood analysis is very easy to perform and is an objective estimate of fetal biochemistry. The percentage of babies born with severe acidosis in a vigorous state is certainly greater than most clinicians would predict. It is reasonable to consider that severe metabolic acidosis in the fetus reflects oxygen deprivation and the likelihood of asphyxial brain damage. Assessment of the predictive value of cord blood results and neurological damage is beyond the scope of this study. The clinical utility of completing cord blood analyses will depend on additional research with long-term neurological and developmental assessment of acidotic and normal children. However, we can safely state that routine umbilical cord blood pH analysis is not clinically helpful in early neonatal evaluation and management.

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Cervical endometriosis: Facilitated diagnosis by fine needle aspiration cytologic testing

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Regarded as infrequent, endometriosis of the cervix probably occurs more commonly than is generally realized. The lesion's apparent rarity may be ascribed to limited awareness of the clinical appearance, combined with technical difficulty in obtaining suitable biopsy material for confirmation. A consequence of previous cervical trauma, endometriosis of the cervix can be expected to increase in incidence with more widespread use of invasive cervical procedures. Clinical recognition and tissue confirmation thus become essential. This paper refocuses attention on cervical endometriosis by documenting the clinical, colposcopic, and histopathologic findings in 16 cases seen at a single colposcopy clinic over a 6-month period. Diagnosis in these patients was facilitated and improved by fine needle aspiration cytologic testing. This technique, not previously used for documentation of endometriosis in this location, is outlined in detail, together with a full description and illustration of the cytologic features. (Am J Obstet Gynecol 1987;157:849-56.)

Key words: Aspiration, cytology, cervical endometriosis

Endometriosis of the cervix is usually regarded as an uncommon lesion, most gynecologic texts making only brief mention of its existence. After an initial observation by Fels[1] in 1928, only 12 single case reports had accumulated in the literature by 1952, when Ranney et al.[2] challenged the apparent rarity of the lesion with a series of 16 cases seen in a 4-year period. Further supportive evidence was provided by Williams,[3] who documented 111 cases in 1960. Both Ranney and Chung[2] and Williams[3] attributed the dearth of reported cases to lack of clinical awareness of the lesion, difficulty in obtaining adequate biopsy material, and poor recognition of the histologic features.

The classic "endometrioma" of the cervix is an obvious round, raised, hemorrhagic lesion, readily visible on speculum examination and easily sampled. Many of the lesions described by Ranney and Chung and by Williams were, however, small, nonprotuberant, and fragile. Superficially located on the tough, fibrovascular cervical stroma of the ectocervix, they were easily overlooked or readily ruptured and traumatized during attempts at punch biopsy. The resultant crushed tissue, containing a few fragmented glands or only sheared stroma, was often not recognized as being endometrial in type by the examining pathologist.

Development of endometriosis in this location is ascribed to previous cervical trauma, which was documented in 75% and 95%, respectively, of the cases of Ranney and Chung and of Williams. With increasing use of diagnostic and therapeutic traumatizing cervical procedures, as well as extended use of the colposcope since that time, a definite increase in reported cases of cervical endometriosis might have been expected. However, apart from smaller series published by Wolfe et al.[4] in 1961 and by Gardner[5] in 1962, no such documentation has been found in the English literature. Of all colposcopy texts consulted, only one, by European authors Dexeus, Carrera, and Coupez,[6] was found that alludes to and illustrates cervical endometriosis. It would thus appear that the previously disproved myth regarding the rarity of this lesion has resurfaced.

The purpose of this communication is twofold: to reintroduce the neglected topic of endometriosis of the cervix into the English literature by documenting 16 cases seen at a single colposcopy clinic over a 6-month period and furthermore to propose, for the first time, a new method of diagnosis—that of fine needle aspiration cytologic testing, which in our experience has provided a rapid, accurate, and relatively noninvasive method of confirmation of clinically and colposcopically suspicious endometriotic lesions of the cervix.

Patients and methods

Sixteen patients with verified cervical endometriosis, who form the basis of this report, were all seen at a

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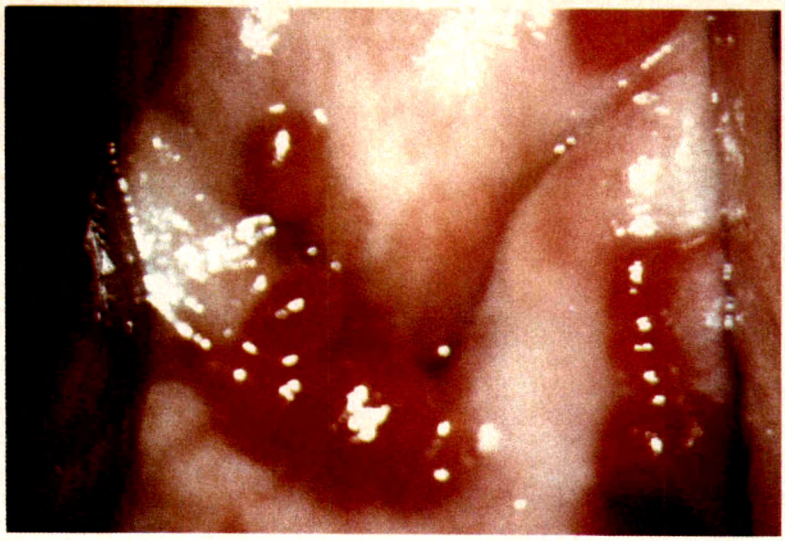


Fig. 1. Colpophotograph of cervical endometriosis seen 3 days before menstruation. Note multiple, irregularly shaped but well-delineated, raised, red, domed, submucosal nodules on the ectocervix. One large focus abuts on the external os but does not extend into the cervical canal. (Original magnification ×1.4.)

single colposcopy clinic during a 6-month period, during which a total of 670 patients underwent colposcopic examination by one author (M. M. V.-F.). Indications for colposcopic evaluation were abnormal cervical smears in nine, vaginal discharge in four, abnormal vaginal bleeding in three, and follow-up after previous conization in two. In all 16 patients, ectocervical lesions were identified either macroscopically or colposcopically and were strongly considered clinically to be endometriotic foci. In the first two patients seen, directed biopsies with standard punch biopsy forceps failed to provide histologic confirmation. In an attempt to obtain verification of the lesions seen, the technique of fine needle aspiration (previously successfully applied by the same colposcopist in two cases of rectovaginal endometriosis[7]) was performed by use of a standard 20 ml syringe and 21-gauge needle. The needle tip was introduced superficially into the cervical lesion, and gentle suction was applied by withdrawal of the plunger with the needle still in situ. The syringe and needle were then withdrawn, and the needle contents expelled and spread onto a plain glass slide, with a spray fixative used for rapid fixation. A small amount of hemorrhage occurred at the needle puncture site. This was used as a guide for immediate biopsy of the focus, which was generally less protuberant after needle aspiration. Subsequent bleeding from the biopsy site was not profuse in any of the cases and was controlled by direct pressure. In the laboratory, aspiration smears were stained by the Papanicolaou stain. The biopsy specimens were aligned, embedded in their entirety, and initially sectioned at two levels. This was soon shown to be inadequate, and ultimately an average of 12 levels was cut and examined from each specimen. All histologic specimens were stained with hematoxylin and eosin.

Initial colposcopic evaluations were performed during menstruation. Once the diagnosis of endometriosis was established, the patients were encouraged to return before or during menstruation. A total of 41 evaluations was performed on these 16 patients, of whom only three were not seen around the time of menstruation, their examinations being at midcycle.

Results

The 16 patients with cervical endometriosis represented 2.4% of patients undergoing colposcopic evaluation during that time period. All were black, their ages ranging from 23 to 45 (average 31). Within the group parity ranged from 0 to 5 (average 2.5). Of the total, 56% had previously documented cervical procedures, including cone biopsy in two, cervical curettage in six, laser therapy in three, and electrocautery in one.

Colposcopic findings. Marked variability was noted in the appearances of endometriotic foci of the cervix. All were characterized by a definite ectocervical location, with no encroachment on the squamocolumnar junction, even if close to the external os of the cervix. The lesions were submucosal, covered by epithelium with smooth, shiny characteristics of squamous epithelium, as opposed to red, granular appearances of endocervical epithelium, seen colposcopically (Fig. 1).

Size, shape, and color were evanescent, progressive, or regressive, depending on the stage of the menstrual cycle. In the three patients in whom examination was



Fig. 2. Colpophotograph of same cervix illustrated in Fig. 3. Seen at midcycle, the endometriotic foci are macular, hemorrhagic, small lesions situated on the ectocervix. (Original magnification × 1.4.)



Fig. 3. Colpophotograph of cervix illustrated in Fig. 2, seen 12 days later, 2 days before menstruation. The hemorrhagic areas remain flat but have spread to involve large areas of the ectocervix, sparing the area around the external os. (Original magnification × 1.4.)

possible only at midcycle, the lesions were macular and plaque-like, just slightly elevated from the contour of the ectocervix. In all patients seen around menstruation, the lesions were seen to enlarge, extend, and become dome-shaped, cystic, or nodular. The majority were round, but linear and serpiginous foci, generally with well-defined contours, were also seen. Toward the end of the menstrual cycle, individual lesions became confluent and in one patient assumed a widespread appearance, involving almost the entire ectocervix (Figs. 2 and 3). This was the only lesion that became ulcerative when seen after menstruation. The lesions were red or red-blue in color and acquired a mottled appearance close to menstruation. The smallest lesion seen measured <2.0 mm in diameter and was well visualized only through the colposcope. The average lesion size was 1.0 cm intermenstrually, extending to a diameter of 2.0 cm at maximum development. The larger lesions were easily seen macroscopically as erythematous areas. Neither scarring nor puckering was observed in any of the cases, and in none did the lesions extend into the vaginal fornices.

Cytologic appearances. In 10 of 14 patients on whom needle aspiration was performed, cellular features di-

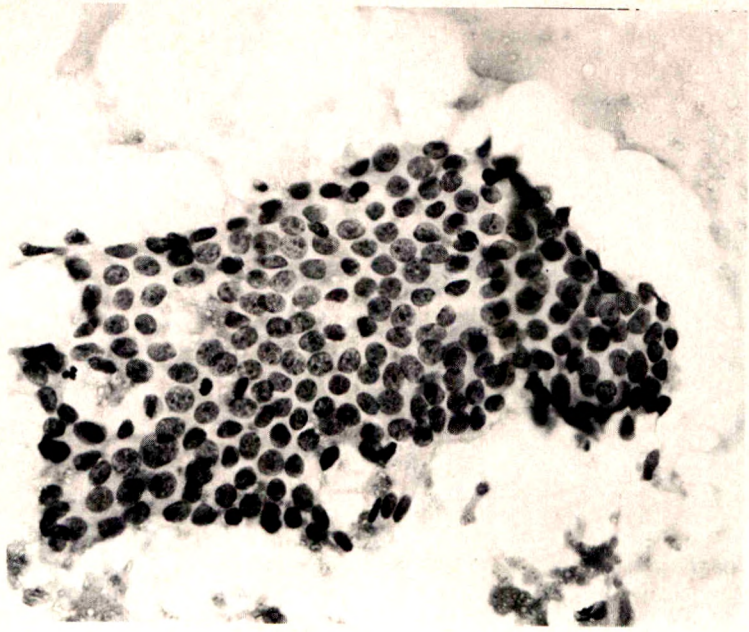


Fig. 4. Endometrial glandular cells in fine needle aspiration cytologic smear. Round-to-oval monomorphic cells form a monolayer sheet with poorly defined cytoplasmic borders. (Papanicolaou stain. Original magnification ×500.)

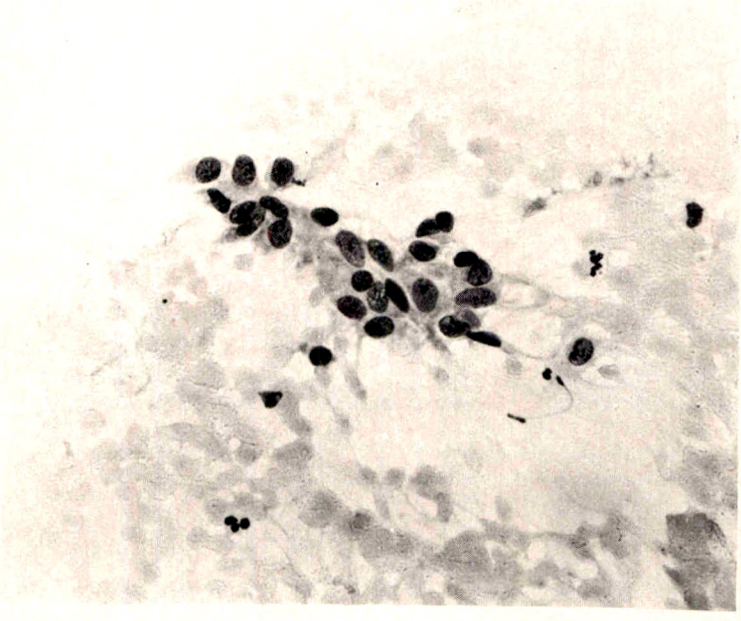


Fig. 5. Endometrial stromal cells in fine needle aspiration cytologic smear. Cells are elongated with abundant basophilic cytoplasm. Cytoplasmic borders are poorly defined in the loose aggregate. (Papanicolaou stain. Original magnification ×500.)

agnostic of endometriosis were found and provided the first confirmation of the clinical impression. Both endometrial glandular and stromal cells were present in eight of these 10 aspirates; one contained identifiable stroma only; one showed only glandular cells. In the remaining four cases, only blood and squamous cells from the overlying epithelium were seen. These four cases had verification on histologic examination. Endometrial glandular cells in needle aspiration specimens occurred in monolayer sheets (Fig. 4) or in small, more irregular clusters. In the flat sheets, nuclei were evenly dispersed, round, even, and vesicular, with small

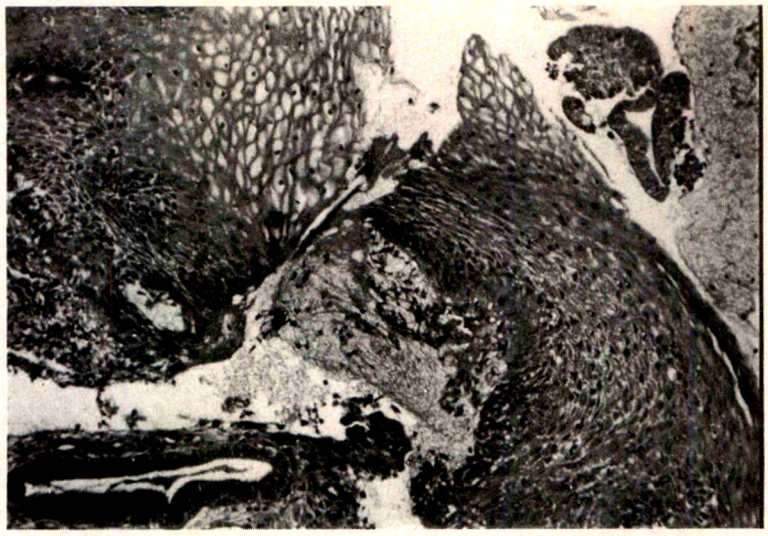


Fig. 6. Histologic section from a case in which the fine needle aspiration cytologic smear was nondiagnostic. The biopsy specimen was traumatized by the fine needle aspiration needle track extending through the ectocervical squamous epithelium into an endometriotic focus *(bottom left)*. Two clusters of aspirated endometrial glandular cells remain adjacent to the surface epithelium *(top right)* after failed suction. (Hematoxylin and eosin. Original magnification ×200.)

chromocenters and occasional small nucleoli. Sheets of this nature were distinguished from endocervical cells, which they resembled, by their smaller size and by absence of well-defined cytoplasmic borders. In the smaller groups, nuclear features were identical, but crowding resulted in nuclear molding and loss of polarity.

Endometrial stromal cells appeared as large syncytial fragments or as smaller groups of spindle-shaped cells, with more abundant cytoplasm than noted in glandular cells. Cytoplasmic outlines were poorly defined, but staining characteristics were uniformly basophilic. Some pleomorphism in nuclear size and shape ranging from oval to more spindle-shaped could be appreciated. Where well preserved, chromatin was granular but evenly dispersed (Fig. 5). Siderophages were seen in only four of the cases and were not required for definitive diagnostic purposes.

Sensitivity of diagnosis by fine needle aspiration cytologic testing was 71%, with 100% specificity. Absence of diagnostic cellular material was probably related to technical problems, as evidenced by a case illustrated (Fig. 6). No endometrial glandular or stromal cells were seen in the cytologic preparation. The biopsy performed simultaneously, however, showed the needle track leading to a submucosal focus of endometriosis. A cluster of cells, which had obviously been successfully aspirated from this focus, was seen in the section lying just above the ectocervical squamous epithelium. This in all probability resulted from the suction being released too suddenly, with loss of the cells from the needle.

Histologic features. A total of 23 biopsies was performed on various occasions on these patients. On routine examination, when two standard levels were cut from each block, only six of these cases were reported as showing histologic endometriosis. However, when aspiration cytologic results became known and the blocks were subjected to multiple serial sections, seven further lesions were found. The initial sensitivity of biopsy diagnosis was thus 26% with a final review sensitivity (alerted by cytologic results) of 57%.

The histologic features of endometriosis in these cases differed from that described for the classic "endometrioma" of the cervix mainly in terms of lesion size. Many foci measured no more than 2 to 3 mm in diameter or in depth, thus requiring multiple levels to ensure adequate demonstration. Initial sections frequently showed only a few red blood cells lying beneath the basement membrane of the ectocervix. With deeper sampling, characteristic endometrial stroma and glands became evident. The endometrial glands were lined by a single layer of cuboidal closely packed cells. Confusion with endocervical cells was obviated by marked ectocervical location and by absence of the neatly palisaded, tall-columnar appearance characteristic of endocervical cells. Glands were embedded in a stroma of varying cellularity, characterized by small round or oval cells and easily distinguished from the surrounding cervical stroma, which presented a less cellular, more fibrous appearance (Fig. 7). Areas of hemorrhage were frequently observed, but proliferative or secretory changes were less readily apparent. The needle track was usually incorporated in the biopsy specimen. One particular

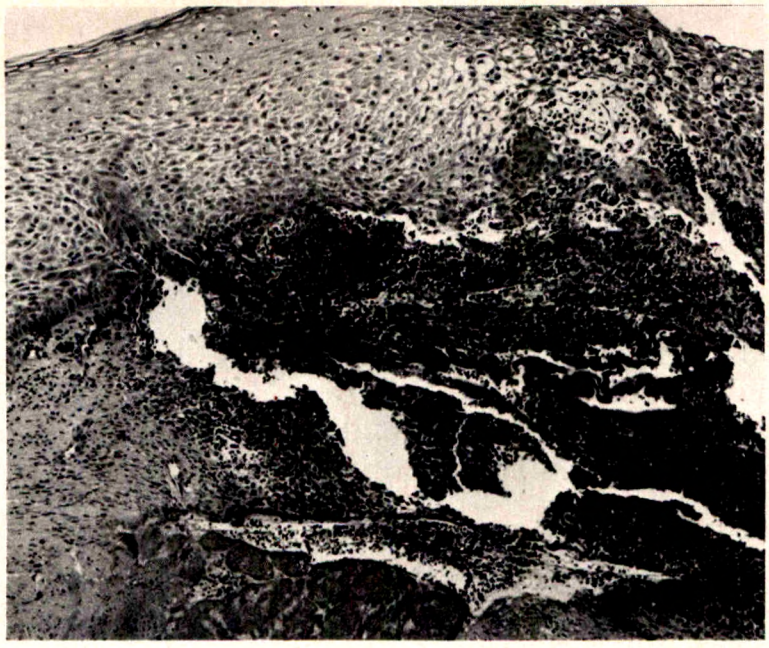


Fig. 7. Low-power view of cervical endometriosis traumatized by previous aspiration. The needle track is seen on the extreme right of the picture. Beneath the ectocervical squamous epithelium, cellular endometrial stroma and a single gland are seen. The endometrial stroma is readily distinguished from the more fibrous ectocervical stroma. (Hematoxylin and eosin. Original magnification ×125.)

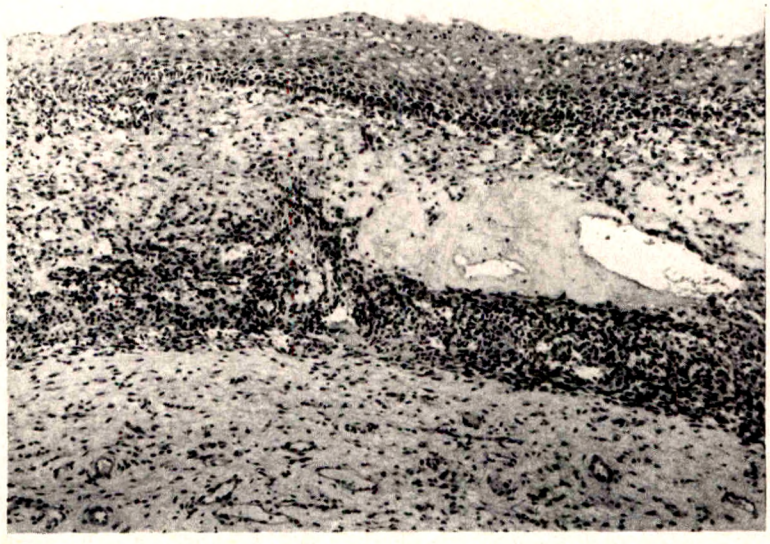


Fig. 8. Low-power section of case initially misinterpreted as showing cervicitis. A band of high cellularity lies between the basement membrane of the ectocervical squamous epithelium and the dense ectocervical stroma. Deeper sectioning revealed the picture shown in Fig. 9. (Hematoxylin and eosin. Original magnification ×125.)

lesion warrants further comment. The specimen was taken from the patient mentioned above in whom a spreading lesion encompassed almost the entire ectocervix before menstruation. This biopsy specimen had been misinterpreted as showing marked inflammatory changes at the time of first examination. After endometriosis was found on aspiration cytologic testing, this lesion was reexamined. As seen (Fig. 8), the initial

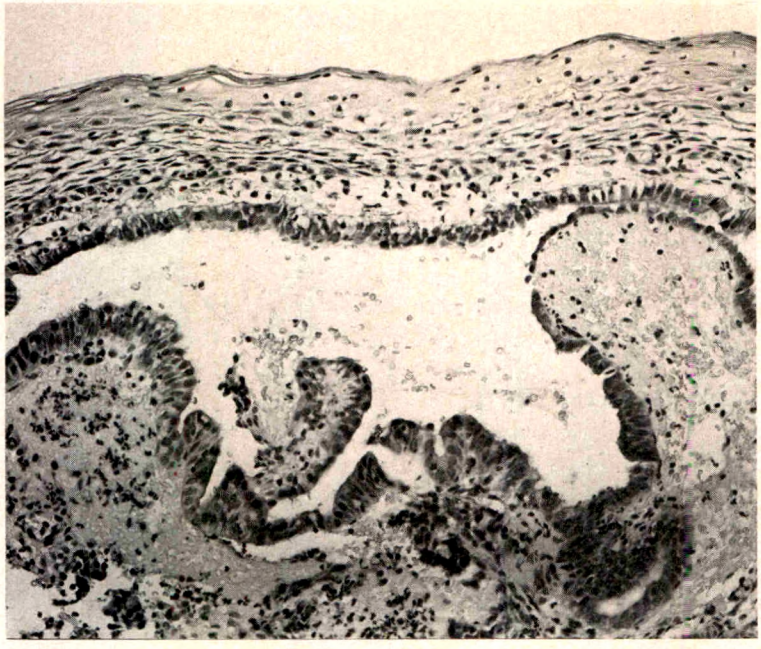


Fig. 9. Deeper sectioning of biopsy specimen shown in Fig. 8 shows endometrial glands immediately subjacent to the basement membrane of the squamous epithelium. (Hematoxylin and eosin. Original magnification ×200.)

impression is of a marked inflammatory infiltrate beneath the ectocervical epithelium. However, close inspection reveals that the band of cellular material consists of endometrial stroma and not of inflammatory cells. Deeper sections taken from this block subsequently demonstrated well-formed endometrial glands within this cellular stroma (Fig. 9).

Comment

Despite the pioneering work in the 1950s and 1960s,[2, 3, 8, 10] it would seem that the concept of cervical endometriosis as a not infrequent occurrence has been lost to later generations of gynecologists, colposcopists, and histopathologists. For this reason, almost 60 years after its first description,[1] it is still not possible to quote the incidence of this condition, nor is it known whether its frequency is rising, as might be expected with greatly extended use of cervical procedures such as conization, colposcopic biopsy, diathermy, and laser therapy. The only incidence figures quoted are by Dexeus et al.,[6] who tabulated the number of cases of cervical endometriosis observed in six European colposcopic series. The estimates ranged from 0.11% to 0.44%, but neither the populations nor the reasons for colposcopy were further defined. In our series of 670 black patients undergoing colposcopic evaluation for abnormal cervical smears, vaginal discharge, abnormal bleeding, or follow-up after conization, 16 had cervical endometriosis proven by aspiration cytologic testing, histopathologic examination, or both. This incidence of 2.4%, significantly higher than any previous estimate, is felt to be a consequence of heightened clinical awareness of the condition, together with intensified pursuit of a procedure whereby tissue confirmation could be obtained in the face of initial biopsy failures.

Fine needle aspiration of suspicious, elevated cervical nodules has, in this small series, been shown to facilitate and improve pathologic documentation. More extensive sectioning than is usual in laboratory specimens, prompted by cytologic findings, raised the sensitivity of histologic confirmation from 26% to 57%. The poor performance of punch biopsies in the study mirrors the experience of early authors. Ranney and Chung[2] noted that biopsies should be performed without causing trauma or crushing, and even with the best-quality tissue, the individual interpreting the slides should be "endometriosis conscious." Williams[3] observed that biopsy instruments that depended on crushing, shearing, or scraping would almost always fail to secure satisfactory tissue except from relatively large lesions. He advised that cold knife biopsy of suspicious lesions was the preferred method for obtaining diagnostic tissue.

Problems in obtaining optimal material can be obviated by utilizing fine needle aspiration cytologic testing as shown in this series. The ability to diagnose endometriosis in aspirated cytologic material from nonfluid foci has previously been documented in aspirates from endometriotic scars[9] and from the rectovaginal septum.[7] The association of small columnar cells in monolayer sheets or crowded clusters, together with more elongated stromal cells, is characteristic and specific. The sensitivity of cytology in this series, at 71%,

was higher than that of histopathologic diagnosis. Cytology also provided the first confirmation of many clinically suspicious lesions, thereby promoting a more intensive search for histopathologic verification.

Queries relating to the necessity for cell or tissue confirmation of what is apparently a recognizable and specific clinical lesion were answered by Williams,[3] who noted that endometriotic foci may be mimicked by hemorrhagic nabothian cysts, cervicitis with focal hemorrhage, hemangiomas, decidual reactions, and even malignant lesions. All these can readily be differentiated from endometriosis by an "endometriosis conscious" pathologist, and none would produce the characteristic dual cell population obtained by fine needle aspiration from a focus of endometriosis. In support of this, during this study period, two further patients were seen with colposcopic appearances suggestive of endometriosis. Cytologic results were negative in both cases. On histologic examination, one lesion was a hemorrhagic nabothian cyst, and the other contained postradiation petechiae. These examples highlight the need for cytopathologic diagnosis.

The clinical significance of endometriosis of the cervix has been addressed by Gardner and Kaufmann,[10] in whose experience it was considered one of the chief causes of persistent or recurrent minimal metrorrhagia, in particular, causing perimenstrual spotting and contact bleeding. Only two patients in the current series complained spontaneously of abnormal bleeding. The cervical lesions in all patients were isolated findings. None had evidence of internal pelvic or extrapelvic endometriosis on clinical examination. This is in keeping with the experience of all other authors. The fact that cervical foci in these patients appear to be isolated or "primary," without coexistent pelvic stigmas, as well as the known relationship of this lesion to previous cervical procedures, lends strong support to Sampson's theory of implantation as an etiologic mechanism.

Treatment remains problematic. If, as has been stated, cervical endometriosis is a potent source of intermenstrual bleeding, then treatment is clearly desirable. On the other hand, if its very existence is related to surgical procedures, further locally destructive therapeutic mechanisms such as excision or fulguration would create a potential for reimplantation and recurrence. While Williams and Richardson[8] claim successful treatment by light cauterization, Gardner and Kaufmann[10] document "surprisingly frequent" recurrence. No data are available on treatment by newer modalities such as cryotherapy or laser therapy.

More than half a century after its first description, cervical endometriosis remains poorly documented and little understood. In refocusing the attention of gynecologists and pathologists on this topic, we hereby wish to promote the use of fine needle aspiration cytologic testing as an inexpensive, atraumatic, sensitive and rapid method of diagnosis—a method that can avoid the difficulties of biopsy confirmation in many cases. The technique will, however, be of value only if the examining clinician and the pathologist maintain a high index of suspicion and redevelop an awareness of both the existence and appearance of cervical endometriosis, well documented many years ago.

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A novel Y/13 familial translocation

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A novel Y/13 translocation was discovered in a female fetus during amniocentesis. The familial translocation is present in a phenotypically normal female sibling and in the father who also possesses a pericentric inversion. Karyotype of the father is 46,XY, − 13, + der(13)t(Y;13)(q12;p12),inv(22)(p13q12.1).
(AM J OBSTET GYNECOL 1987;157:857-8.)

Key words: Y chromosome, autosome 13, translocation

Reports of translocations between the Y chromosome and autosomes (numbers 13, 14, and 15) of the D group have accumulated slowly over the past 15 years. The frequency of Y/15 translocations has been found to greatly exceed the combined occurrence of Y/14 and Y/13 exchanges.[1] The incidence of the Y/14 type approximates that of the Y/13 type.

Although less than 10 cases* of Y/13 translocation have been reported, the nature of chromosomal transposition and the consequence of clinical manifestation vary among them. Nearly all these cases involved males who had diverse phenotypic expressions, such as subfertility, mental retardation, encephalocele, bilateral clubfoot, or a variety of other congenital anomalies. Karyotypes of most of these patients were either unbalanced or incompletely ascertained because of an inability to specify exact breakpoints in the translocated chromosomes. Reports of Y/13 translocations in females with completely documented karyotypes are extremely rare and appear to be limited to a single case described by Vianello et al.[2]

Case report

A routine amniocentesis that was performed during late 1986 on a patient at our prenatal diagnosis facility at the University of California, Davis, Medical Center resulted in a fetal karyotype believed to be of novel designation: 46,XX, − 13, + der(13)t(Y;13)(q12;p12)pat. Single cells from 15 colonies of amniocytes all revealed the same karyotype (under GAG/GTG methods), which indicates that breakpoints occurred at bands q12 and p12 in the long and short arms of chromosomes Y and 13, respectively. Segments distal to these bands have been reciprocally exchanged between the two chromosomes. The use of NOR, CBG, and QFQ banding techniques showed that the additional material on the p arm of one number 13 autosome is C and Q band positive and NOR negative. Thus the brightly fluorescent Q positive material indicates the presence of Y material on autosome 13. Because this Y/13 translocation is also found in the clinically normal father of the fetus (mother is 46,XX), as well as in a normal 1-year-old female sibling, the karyotype is considered a familial variant and probably lacks clinical significance, although sexual development in both the infant and impending neonate will be followed. The father's genome also contains a pericentric inversion in chromosome 22. Thus this novel karyotype can be designated as 46,XY, − 13, + der(13)t(Y;13)(q12;p12),inv(22)(p13q12.1).

Comment

This study differs from that of Vianello et al.[2] in at least three aspects. First, our study confirms that the breakpoint in autosome 13 is at band p12, whereas Vianello et al.[2] indicate that the autosome 13 breakpoint could be either p11 or p12. Second, in their study, the father of the patient lacked the inversion identified in the father of our patient. Third, Vianello et al.[2] reported a complete palatolabioschisis, low-set ears, and a malformation of the right ear in their Y/13 female, whereas our Y/13 patients have been adjudged to be clinically normal.

The presence of the Yq12 segment in a normal female appears to have little effect on sexual development, and the prognosis for normal sexual development in both the fetus and 1-year-old patient may be considered good. The reason for this is that the Yq12→Yqter portion of the Y chromosome consists of constitutive heterochromatin, which lacks genes for maleness. Thus male-determining factors on the Y chromosome probably reside outside of the Yq12 region.

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**A complete set of references is available on request.*

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In vitro training model for diagnostic and therapeutic fetal intravascular needle puncture

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An in vitro model, simple to assemble, consisting of a fresh placenta suspended in a water bath, is presented for the training of physicians interested in practicing ultrasound-guided fetal intravascular needle placement. (Am J Obstet Gynecol 1987;157:858-9.)

Key words: Fetal blood sampling, fetal intravascular procedures, ultrasound-guided needle puncture

Ultrasound-guided fetal blood sampling through the umbilical vein was first introduced by Daffos et al.[1] in 1983.

Direct access to the fetal vascular compartment opens wide possibilities for antenatal diagnosis, therapy, and clinical research.[2]

Despite the large potential, this procedure is used only by a handful of skilled, scientifically oriented clinicians because it requires special skill and experience by the operator. This skill is earned almost entirely by experience, which, in turn, increases proportionately with the growing number of procedures performed. In any given center, there may not be a large enough number of pregnant patients requiring fetal blood sampling or intravascular transfusions, thereby making it difficult to acquire skill and excellence. This, of course, hampers the training of others.

To overcome the problem of physician training, we developed an in vitro model to perform ultrasound-guided umbilical vessel puncture.

Material and method

A recently delivered intact placenta with the cord clamped at 20 cm from its placental insertion was used. Heparin was injected through both the umbilical veins and the artery one at a time and mixed thoroughly with the blood by repeated aspiration and reinjection by means of a 20 ml syringe. At the end of this procedure, the cord was clamped by a Hollister clamp after the

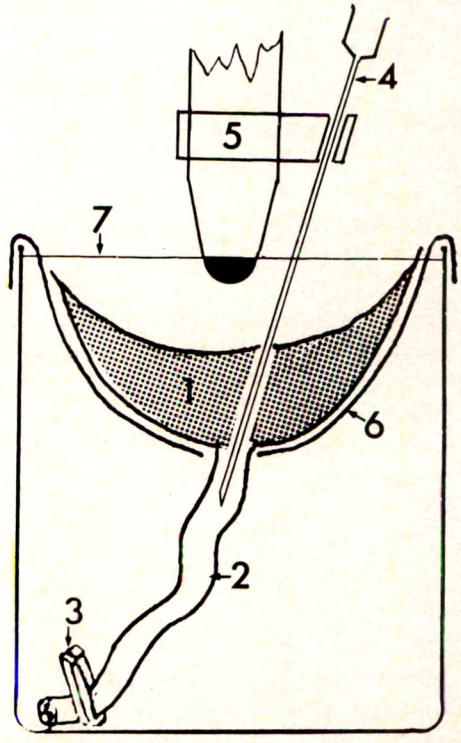


Fig. 1. Schematic illustration of the model. (*1* = Placenta; *2* = umbilical cord; *3* = clamp occluding the cord; *4* = 8- to 10-inch needle; *5* = transducer probe fitted with needle guide; *6* = gauze "hammock," and *7* = water filling the tank.)

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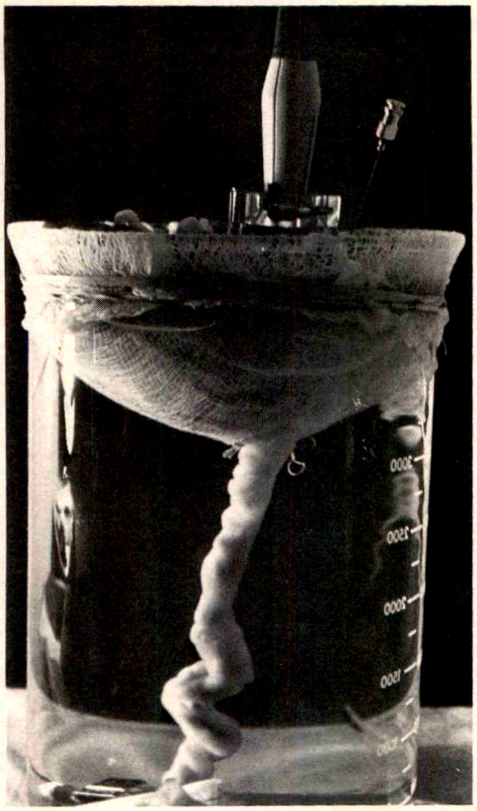


Fig. 2. Placental model.

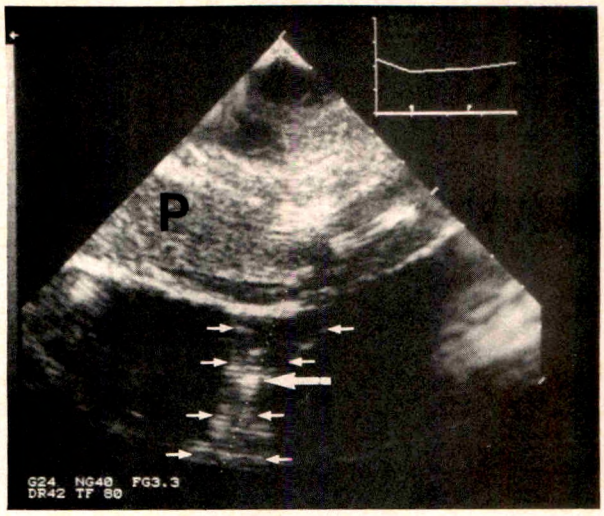


Fig. 3. Ultrasonographic picture of the model. (*P* = Placenta; *small arrows* = umbilical cord; *large arrow* = needle tip.)

fetal aspect of the placenta was inspected to ensure the presence of engorged and blood-filled vessels to represent in vitro anatomy. The placenta was suspended by a gauze "hammock" in a water-filled tank. The water should be left to settle for 30 minutes to get rid of microbubbles that would otherwise interfere with the scanning. The cord was threaded through a small opening in the middle of the "hammock" and fastened to a heavy object to prevent free floating of its clamped end.

The schematic illustration of the setup is shown in Fig. 1. After the tank was filled with additional water, the scanning is started with a sector-scanning probe fitted with the needle guide attachment (Fig. 2). The proper section and direction for needle placement are selected and aligned with the software generated needle path line on the screen. The needle is gently advanced under ultrasound guidance into the target vessel (Fig. 3). An attempt is then made to withdraw blood. If successful, the tip of the needle should be inspected through the transparent glass tank by gently moving it to touch the vessel wall. This movement of the needle tip is visible and enables correlation of the sonographic image with the actual position of the needle. If the vessel wall is punctured and water is aspirated, the site of perforation should be inspected to complement the on screen needle positioning with that in situ. The correct needle placement should be undertaken after this.

A posterior or lateral cord insertion can be simulated by positioning the placenta on the bottom or the lateral wall of the tank with the cord pointing upward or sideways, respectively. A different way to prepare the model is to flush the umbilical vessels and replace the blood with a diluted methylene blue solution. However, we suggest keeping the blood already within the vessel because it presents the authentic feeling of aspirating fetal blood through the thin needle at the time of a true sampling procedure.

One should start with a term placenta and gradually use placentas originating from pregnancies with younger gestational ages. The aim is to be proficient enough to successfully sample fetuses before reaching the gestational age at which an indicated termination of the pregnancy is prohibited by law.

Comment

A fetal blood sampling model was described and suggested for in-service training of perinatologists who wish to become proficient in this method.

The advantages of this model are: (1) It is simple to assemble, (2) it is inexpensive, (3) placental supply is abundant, and (4) no lengthy review by institutional human investigation committees is required.

After the method of intravascular needle placement is mastered, it is possible to use this model and proceed to more complex tasks of transfusing fluid or performing exchange transfusions.

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Antepartum fetal surveillance in the patient with decreased fetal movement

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Whenever a patient has the subjective perception of decreased fetal movement, prompt evaluation in the form of antepartum fetal surveillance has been undertaken. The purpose of this report is to describe our experience with 489 pregnant women who came between Jan. 1 and Dec. 31, 1985 to our Antepartum Fetal Surveillance Clinic with this complaint alone or in association with another indication for fetal surveillance. Overall, 838 nonstress tests were performed, and the results were reactive, 93.2%; nonreactive, 6.8%; and fetal heart rate decelerations, 6.1%. Comparison of the first nonstress test results between those with decreased fetal movement alone or in combination with another diagnosis demonstrated a similar incidence of nonreactivity and fetal heart rate decelerations. In those patients whose indication was decreased fetal movement alone, there was a 3.7 times greater likelihood of diminished amniotic fluid volume. When the last test within 7 days of delivery was analyzed, the decreased fetal movement alone group had a lower incidence of cesarean delivery, cesarean delivery for fetal distress, and Apgar scores <7 than patients with an additional indication for testing. In summary, decreased fetal movement continues to be an acceptable indication for fetal surveillance. Based on our retrospective experience, the most reasonable approach appears to be a combination of nonstress test and amniotic fluid volume assessment. Unless the patient has additional indications for fetal surveillance, the patient with decreased fetal movement with a reactive nonstress test and a normal amniotic fluid volume does not appear to warrant additional testing. (Am J Obstet Gynecol 1987;157:860-4.)

Key words: Decreased fetal movement, antepartum fetal heart rate testing, amniotic fluid volume assessment, fetal acoustic stimulation

Between 16 and 20 weeks of pregnancy, the pregnant woman first perceives fetal movement. Although normal fetal movements are considered a sign of fetal well-being, decreased or absent fetal movement can be a sign of fetal jeopardy.[1] Previous investigators[1, 2] have demonstrated a relationship between decreased fetal movement and fetal distress or impending fetal death. This has been especially true in the high-risk pregnancy.[2] However, Milliez et al.[3] could find no correlation between the absence of fetal movements and subsequent fetal outcome. Nonetheless, these investigators found a less favorable outcome when fetal heart rate accelerations were absent.

However, little information is available concerning the relationship between the results of antepartum fetal surveillance testing and the patient who has the complaint of decreased fetal movement. Therefore the purpose of this retrospective investigation was to describe our experience with antepartum fetal surveillance testing in this group of patients.

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Material and methods

From Jan. 1, 1985 through Dec. 31, 1985, all patients who came to the Antepartum Fetal Surveillance Clinic at the Los Angeles County/University of Southern California Medical Center with the subjective complaint of decreased fetal movement were reviewed. During this period, 489 patients underwent 838 nonstress tests for decreased fetal movement alone or in combination with another indication. Outcome data were based on all patients who delivered within 7 days of their last nonstress test.

All fetal surveillance tests were performed by full-time antepartum-testing nurses trained in ultrasonography and fetal monitoring. These tests primarily included a nonstress test, amniotic fluid volume assessment by means of a four-quadrant technique, or biophysical profile. Results of the fetal surveillance test were confirmed by a Maternal-Fetal Medicine Division staff member or fellow.

Table I. Indications for antepartum fetal surveillance in a group of patients with decreased fetal movement (January to December 1985)

Indication	Overall (N = 184)		Known outcome* (N = 127)	
	No.	(%)	No.	(%)
Postdates	99	53.8	74	58.3
Diabetes mellitus	36	19.6	25	19.7
A	24		14	
B	12		11	
Hypertension	13	7.1	8	6.3
Prior fetal demise	11	6.0	9	7.1
Suspected intrauterine growth retardation	10	5.4	5	3.9
Other	15	8.1	6	4.7

*Delivery within 7 days of the last test.

The procedure, criteria for the performance, and interpretation of the nonstress test have been previously described.[4] During the period of investigation, the primary technique used to establish a reactive nonstress test was transabdominal acoustic stimulation of the fetus with an electronic artificial larynx. In brief, transabdominal stimulation overlying the vertex is done for ≤3 seconds. If the fetus is unresponsive, the stimulus was repeated two additional times for a total of three stimuli. The criteria for a reactive nonstress test were the presence of two or more fetal heart rate accelerations of at least 15 bpm lasting a minimum of 15 seconds within a 10-minute window. Nonreactive nonstress tests were defined when the criteria for reactivity were not met within a 40-minute monitoring period.

If a test was interpreted as nonreactive, a fetal biophysical profile[5] or contraction stress test was used. Fetal heart rate decelerations were defined as a decline of the fetal heart rate from baseline of 15 bpm lasting 15 or 30 seconds if the deceleration immediately followed an acceptable acceleration (15 bpm × 15 seconds).

Amniotic fluid volume was assessed by the four-quadrant technique or amniotic fluid index previously described by Phelan et al.[6] In brief, the uterus is divided into four separate quadrants with the patient in the supine or semi-Fowler position. The uterus is divided into upper and lower halves by an imaginary line that traverses the umbilicus transversely. Then by using the linea nigra as the midline, the uterus is further divided into right and left halves. The transducer head is placed along the maternal longitudinal axis, perpendicular to the floor. The vertical diameter of the largest fluid pocket is identified and measured in centimeters for each quadrant. The summation of measurements from each quadrant represented the amniotic fluid index in centimeters for each patient. Simultaneously, placental location was determined during ultrasonographic evaluation.

Table II. Nonstress tests results for patients with decreased fetal movement alone (group I) or in combination with another indication (group II)

Nonstress test result	Overall			
	Group I (N = 390)		Group II (N = 448)	
	No.	%	No.	%
Reactive	358	91.8	423	94.4
Nonreactive	32	8.2	25	5.6

Table III. Nonstress test results at the time the patient complained of decreased fetal movement

Nonstress test result	No.	%
Reactive	458	93.7
Nonreactive	31	6.3

Pregnancy outcome was measured by incidences of meconium, fetal distress that required cesarean delivery, Apgar scores <7 at 1 and 5 minutes, abnormal cord positions (nuchal or truncal cord, a true knot, and funic presentation), and perinatal death.

Data were analyzed by dividing the study population into two groups; decreased fetal movement alone (group I) and decreased fetal movement with another indication (group II). Regarding the amniotic fluid index, the patients were subdivided into two groups: 0 to 5.0 cm and >5.0 cm.

Results

During the study period, antepartum fetal surveillance was performed on 489 patients who had a com-

Table IV. Nonstress test results in relation to amniotic fluid index

Amniotic fluid index (cm)	*No.*	*Nonstress test results (N = 589)*			
		Nonreactive		*Fetal heart rate decelerations*	
		No.	*%*	*No.*	*%*
<5.0	22	3	13.6	5	22.7*
>5.0	567	24	4.2	30	5.3

*$p < 0.01$, $\chi^2 = 8.6115$.

Table V. Pregnancy outcome (N = 220) for those patients with decreased fetal movement alone (group I) or in combination with another indication (group II)

	Group I		*Group II*		*Total*	
	No.	*%*	*No.*	*%*	*No.*	*%*
No.	108		112		220	
Cesarean section	14	13.0	32	28.6	46	20.9
Cesarean section-fetal distress	4	3.7	5	4.5	9	4.1
Meconium	35	32.4	32	28.6	67	30.5
Apgar score <7						
1 min	11	10.2*	23	20.5	34	30.4
5 min	2	1.9	4	3.6	6	2.7
Perinatal deaths	0		1	0.9	1	0.5

*$p < 0.05$.

plaint of decreased fetal movement. Of these patients, 220 (45.0%) were delivered within 7 days of their last evaluation. Of the 489 patients, 163 (33.3%) had additional indications for a nonstress test and 112 (69%) delivered within 7 days of their last test. Table I demonstrates the indications for a nonstress test in these patients. Several patients had more than one indication for evaluation. Even though 31% delivered more than 7 days after their last test, the indications for the outcome group were similar to the overall group.

A total of 838 nonstress tests were performed on 489 patients. Of these, 781 (93.2%) were considered reactive. Table II demonstrates nonstress test results for the two groups: decreased fetal movement alone (group I) and decreased fetal movement with another indication (group II). Fetal heart rate decelerations were found in 51 of 838 tests for an incidence of 6.1%. On the day the patient was tested for decreased fetal movement, 93.7% of the nonstress tests were reactive (Table III). There was no significant difference in nonstress test results in terms of reactivity or the presence of fetal heart rate decelerations between the groups.

Table IV illustrates the relationship between the nonstress test results and the amniotic fluid index. In those patients with an amniotic fluid index ≤5.0 cm, nonreactivity ($p < 0.1$) and the presence of fetal heart rate decelerations ($p < 0.01$) were higher. On the day the patient first complained of decreased fetal movement, those patients whose only indication for testing was decreased fetal movement had a 3.7 times (3% versus 11%) greater likelihood of a diminished amniotic fluid volume.

Placental location was recorded during each ultrasonographic evaluation. On review, no significant difference between anterior and posterior placental locations was found between the two study groups.

The pregnancy outcomes for the two study groups are summarized in Table V. In those patients with decreased fetal movement alone, the cesarean section rate was lower in group I than in group II (13.0% versus 28.6%). Although the incidence of meconium was comparable for both groups, cesarean section rates for fetal distress were 3.7 versus 6.0% for groups I and II, respectively.

Fetal outcome, as measured by Apgar score and perinatal mortality, showed that group I had a 50% lower incidence of Apgar scores <7 at 1 and 5 minutes than did group II. During the study period, one perinatal death occurred (group II). The patient was a 31-year-old woman, gravida 6, para 5-0-0-5, at 31 weeks' gestation who underwent antepartum surveillance for decreased fetal movement and preeclampsia: the nonstress test was nonreactive. Subsequently, she delivered a 1470 gm male baby by cesarean section for fetal dis-

tress caused by a prolonged fetal heart rate deceleration. Apgar scores were 1, 5, and 7 at 1, 5, and 10 minutes, respectively. At delivery, the infant was noted to have multiple congenital anomalies and died on the eighth day of life.

Comment

Although frequently criticized, the subjective perception of fetal movements is the oldest and least expensive means of assessing fetal well-being. With ultrasonography, fetal movements can be detected as early as the seventh week of pregnancy. The objective and subjective perception methods of fetal movements have been well described by Sadovsky and Polishuk.[7] These movements have been characterized as weak, rolling, or strong. Rolling and strong movements increase progressively until 36 to 37 weeks' gestation. At that time, these movements decline and weak movements increase in frequency. Although the weak movements were still perceptible by the gravida, they were sensed less frequently than the strong or rolling movements.[8] During the third trimester, the normal fetus also exhibited some form of trunk, limb, or respiratory movement 10% of the time. However, diurnal variations,[9] maternal glucose concentrations,[9] and acoustic stimulation[4] may alter the frequency of fetal activity. In general, daily fetal movements continue to increase after 18 weeks' gestation and become maximal between 19 and 38 weeks' gestation. Thereafter, both the number of movements and the maternal perception of those movements decrease until delivery.

Previous methods to assess fetal well-being in cases of decreased fetal movement have primarily been biochemical.[9] However, decreased fetal movement is currently one of the more common indications for antepartum fetal surveillance.[4] Although decreased fetal movement is subjective and varies among pregnant women, a decrease in activity of a previously active fetus may symbolize fetal jeopardy.[7]

Although Sadovsky and Polishuk[1] have emphasized that decreased fetal movement is considered a sign of fetal distress, other investigators[3, 10] have reported cases of absent fetal movement with a normal outcome. O'Leary and Andrinopoulos[11] prospectively evaluated the relationship of the nonstress test and daily fetal movement recording for assessing fetal well-being. They found the presence of daily fetal movement neither sensitive nor specific for the detection of antenatal fetal jeopardy in high-risk pregnancies. These investigators failed to identify a relationship between nonstress test results and prior fetal movement pattern. In the current investigation, nonstress test results were also comparable with those reported by O'Leary and Andrinopoulos.[11]

Recent attention has focused on the importance of amniotic fluid volume assessment as an indicator of fetoplacental function[12] and fetal well-being.[13] With the use of real-time ultrasonography, several investigators have demonstrated a relationship between a decrease in amniotic fluid volume and subsequent fetal outcome.[13, 14]

Rayburn[15] previously hypothesized that a gradual decrease in the total amount of fetal motion after the thirty-second week may relate to a variety of conditions, including decreased amniotic fluid volume. Our investigation, which demonstrated a higher incidence of decreased amniotic fluid volume in the patient who had decreased fetal movement, would support Rayburn's theory. Moreover, when the fetal surveillance findings were normal, the current study demonstrated that a more favorable pregnancy outcome was found in the decreased fetal movement alone group than in those patients with additional indications for testing.

In summary, when a pregnant woman perceives decreased fetal movement, antepartum fetal surveillance is indicated to assess fetal well-being. Based on the current study, both nonstress test and some form of amniotic fluid volume assessment appear warranted. If the nonstress test is reactive, the amniotic fluid volume is normal, and the patient has no other indications for testing, follow-up testing does not appear indicated.

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Validity of intrapartum diagnosis of fetal thrombocytopenia

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Performing a platelet count in fetal scalp blood was hitherto the only method to predict whether the infant of a mother with idiopathic thrombocytopenic purpura will be thrombocytopenic. Fifteen such cases are reported. Falsely low platelet counts were found in almost half of these fetal scalp samples. In two patients this could have altered the obstetric management. (Am J Obstet Gynecol 1987;157:864-5.)

Key words: Idiopathic thrombocytopenic purpura, fetal scalp blood, platelet count

Idiopathic thrombocytopenic purpura is an immunologically mediated destructive thrombocytopenia. Maternal autoantibodies can cross the placenta and cause thrombocytopenia in the fetus. Several investigators have tried to find parameters to predict the occurrence and the degree of neonatal thrombocytopenia in order to decide on the less traumatic route of delivery. However, a relationship between the number of maternal and neonatal platelets could not be found. Also data on the correlation between the quantity of free and platelet-bound maternal autoantibodies, on the one hand, and the number of fetal platelets, on the other hand, are conflicting. Platelet counts in a micro

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Table I. Fetal, umbilical cord, and neonatal platelet counts in 15 pregnancies associated with idiopathic thrombocytopenic purpura

Patient identification	*Cervical dilatation at fetal blood sampling (cm)*	*No. of platelets × 10⁹/L*			*Difference between Nos. 1 and 3*	
		1. From fetal scalp blood	*2. From umbilical cord blood*	*3. In neonate immediately post partum*	*No. of platelets × 10⁹/L*	*%*
14B	5	50	290	290	−240	83
16A	4	58		196	−138	70
21A	9	87		249	−162	65
31A	4	60		135	−75	55
III	2	40	127	136	−87	69
X	7	22		109	−87	80
3A	4	186		226	−40	18
10B	4	90	119	104	−14	13
11A	6	121		114	−7	6
12B	3	200	212	200	0	
18B	3	145		104	+41	39
23B	4	54	49	49	+5	10
24A	7	198		198	0	
32B	4	100		100	0	
35B	4-5	267	372	298	−22	7
	9	320				

blood sample obtained from the fetal scalp have therefore been advocated as a method to identify the thrombocytopenic fetus.[1, 2]

Fetal scalp blood platelet counts were performed as part of a collaborative Dutch study on idiopathic thrombocytopenic purpura and pregnancy. Data on fetal and neonatal blood are available from 15 pregnancies. In order to prevent measuring errors due to adhesion of platelets to the sampling devices, the participating centers (n = 10) were instructed to use siliconized glass or plastic pipettes for fetal blood sampling, to transfer the blood immediately to a reservoir containing ethylenediaminetetraacetic acid, and to measure the number of fetal and/or neonatal platelets in an electronic blood cell counter. All gynecologists were experienced with the technique. Patients in whom clotting of the blood sample occurred were not included.

The data obtained are summarized in Table I. A difference of more than 35% was seen in seven of the 15 patients. Platelet counts were more than 50×10^9/L lower in fetal scalp blood than in neonatal blood in six of these cases. Previous publications on this subject show data for a total of 27 patients with idiopathic thrombocytopenic purpura and eight healthy pregnant women.[1, 2] In 11 of these cases there was a difference in platelet count of more than 50×10^9/L between fetal scalp blood and neonatal blood, with 10 of the fetal scalp samples yielding falsely low values and one a falsely high value. In the latter case platelet counts were above 250×10^9/L both in fetal scalp blood and in neonatal blood.[1] Our data and those from the literature hence show that in adequately sampled and analyzed fetal scalp blood, the thrombocytopenic fetus will be correctly identified. As to the falsely low values, two of the six in our series and none in the literature were below 50×10^9/L, the lower limit above which vaginal delivery is generally considered safe. This means that in two of the 15 patients from our series the obstetric management could have been altered unjustly because of falsely low fetal scalp platelet counts.

We conclude that the technique remains clinically useful to exclude thrombocytopenia in the child to be born but that falsely low values with consequences for obstetric management may occur in a small percentage of cases.

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Effects of maternal glucose infusion on fetal acid-base status in human pregnancy

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The maternal and fetal metabolic effects of three commonly used intravenous fluids administered before regional anesthesia were studied in 32 gravid women undergoing elective cesarean section at term. Patients were randomized into one of three groups to receive 1 L of either 5% dextrose (50 gm of glucose) or Ringer's lactate or isotonic saline solution before epidural anesthesia. Acute glucose infusion resulted in maternal hyperglycemia, hyperinsulinemia, and an increase in the blood lactate level. Cord blood glucose, insulin, and lactate levels were also increased in this group. The key finding of this study was the significant lowering of pH in the umbilical cord vein (7.31 ± 0.04) and artery (7.21 ± 0.06) in the glucose-infused group when compared with the non–glucose infusion groups ($p < 0.05$). Confounding perinatal factors such as maternal position, maternal hypotension, and prolonged time of surgery did not influence the fetal acid-base status. Thus acute maternal glucose infusion in normal patients can cause fetal hyperglycemia, metabolic acidosis, and neonatal hypoglycemia. These findings may be of particular clinical importance when fetal distress or fetal hypoxemia is due to other perinatal events. Under these circumstances, acute maternal glucose infusion may further contribute to fetal metabolic acidosis.
(Am J Obstet Gynecol 1987;157:866-73.)

Key words: Pregnancy, intravenous fluids, glucose, fetal acidosis, hyperglycemia

Acute intravenous fluid administration has been recommended to prevent maternal hypotension by expanding volume during regional anesthesia.[1,2] Although this "prophylactic" hydration can be accomplished with various intravenous solutions, acute administration of fluids containing glucose can result in maternal and fetal hyperglycemia and hyperinsulinemia.[3,4] In well-oxygenated fetal lambs, sustained fetal hyperglycemia resulted in increased fetal lactic acid and decreased blood pH. More marked hyperglycemia caused severe metabolic acidosis.[5] Philipps et al.[6,7] demonstrated that sustained fetal hyperglycemia decreased fetal arterial oxygen concentration and induced a state of accelerated fetal oxidative metabolism. Direct insulin infusion to the fetus increased umbilical glucose uptake and decreased fetal arterial oxygen concentration.[8] In contrast to these chronic animal experiments, acute infusion of insulin increased fetal glucose uptake and utilization but did not alter fetal oxygen consumption.[9] Therefore it appears that chronic hyperglycemia and hyperinsulinemia influence glucose uptake, arterial oxygen concentration, and pH, whereas the effects of acute hyperglycemia may not be similar.

In human pregnancy, Kenepp et al.[10] reported that rapid infusion of large amounts of dextrose may be harmful to the fetus and of no benefit to the mother. In their study of women undergoing cesarean section, acute maternal glucose infusion (57.5 gm) resulted not only in maternal and fetal hyperglycemia and hyperinsulinemia but also in a significant decrease in fetal pH. However, intrapartum and perinatal confounding factors other than maternal glucose could have influenced fetal acid-base status. Placental hypoperfusion, as a result of maternal hypotension or maternal supine position, can cause lactic acidosis and influence neonatal acid-base balance at birth.[2,11] In addition, prolonged surgical intervals, that is, the time between induction of labor and delivery of the fetus or the time from myometrial incision to delivery, can influence the incidence of neonatal acidosis and low Apgar scores.[12,13] Therefore the purpose of this prospective randomized study was: (1) to evaluate the maternal and fetal metabolic effects of three intravenous fluids commonly used to prevent maternal hypotension during regional anesthesia and (2) to examine intrapartum perinatal factors that could influence fetal acid-base status.

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Table I. Maternal and neonatal characteristics of the three groups

	Group 1: Glucose (N = 12)	*Group 2: Ringer's lactate (N = 11)*	*Group 3: Normal saline (N = 9)*
Maternal characteristics			
Age (yr)	25.3 ± 5.6	25.8 ± 4.7	25.3 ± 5.1
Gravidity	2.8 ± 1.3	3.1 ± 1.0	3.6 ± 1.0
Parity	2.0 ± 1.2	2.1 ± 1.0	2.1 ± 1.0
Height (cm)	28.6 ± 1.0	28.6 ± 1.0	28.4 ± 0.9
Weight (kg)	73.6 ± 25.9	79.8 ± 13.3	85.8 ± 22.8
Hematocrit (%)	34.1 ± 4.0	35.6 ± 3.3	34.8 ± 2.7
Neonatal characteristics			
Birth weight (gm)	3245 ± 398	3242 ± 322	3383 ± 454
Dubowitz results (wk)	39.1 ± 1.3	38.8 ± 1.3	39.1 ± 1.7

Values are mean ± SD.

Methods and material

All patients had normal, singleton, term gestations and were scheduled for elective cesarean section at Cleveland Metropolitan General Hospital. Patients with liver or kidney disease or any medical complications were excluded from the study. All patients had a negative screening test for diabetes mellitus. After written informed consent was obtained, patients were assigned by a random table of numbers into one of three groups. Group 1 patients received 1 L of 5% dextrose in water (glucose = 50 gm); group 2 patients received 1 L of Ringer's lactate; group 3 (control) patients received 1 L of normal saline solution. The intravenous fluids were administered rapidly during 20 to 30 minutes. Immediately after the hydration, all patients were maintained on a regimen of intravenous normal saline solution at a rate of 125 ml/hr until surgery and delivery of the baby.

Epidural anesthesia was then induced with 0.5% or 0.75% bupivacaine without epinephrine. All patients were placed on the operating room table in the supine position with left lateral uterine displacement and received oxygen at 4 L/min by nasal cannula. Maternal blood pressure was measured every minute with an Arteriosonde (Kontron Inc., Everett, Mass.) from the time of epidural anesthesia until delivery of the infant and every 5 minutes thereafter. Maternal hypotension (a decrease in the systolic blood pressure of >30 torr from baseline or an absolute level <100 torr) was corrected by incremental doses of intravenous ephedrine (5 to 10 mg) and by increasing the rate of infusion of normal saline solution.

Maternal blood samples (3 ml each) were obtained from an indwelling cannula in a peripheral hand vein. The sampling site was kept patent by a heparin lock. In order to obtain arteriolized samples, the sampling site was kept warm (55° C) by a heating blanket. Maternal blood samples were obtained at the start of and after acute intravenous hydration at 10, 20, and 30 minutes and continued every 10 minutes until delivery. Fetal arterial and venous blood samples were obtained at birth from a double-clamped umbilical cord. Neonatal blood samples were also obtained every 30 minutes for 2 hours by venipuncture or heel stick. Since the amount of blood drawn from the infant was limited, the samples at 90 and 120 minutes were analyzed for glucose only. All blood samples were obtained in heparinized syringes and immediately placed on ice. Plasma was separated and stored at −10° C for later analysis. An aliquot of whole blood was precipitated with chilled 10% perchloric acid for lactate measurement.

The data collected during the study also included the skin–to–delivery time interval and the uterine incision–to–delivery interval. At birth, 1- and 5-minute Apgar scores were recorded. Each infant was weighed and a complete physical examination was performed. The gestational age was determined by a modification of the Dubowitz examination by Ballard et al.[14]

Blood samples were analyzed for glucose, lactate, insulin, ketones (β-hydroxybutyrate), and triglycerides. Maternal samples were analyzed for electrolytes, carbon dioxide, and blood urea nitrogen. The baseline maternal samples and umbilical cord samples were also analyzed for acid-base status (pH, P_{CO_2}, P_{O_2}, and base deficit). Blood gas tensions and pH were measured with a microelectrode system (Model 13031, Instrumentation Laboratory, Lexington, Massachusetts). Plasma glucose was measured by a coupled enzymatic assay with the use of hexokinase and glucose-6-phosphate dehydrogenase on an automated centrifugal analyzer (Baker Instruments, Pleasantville, New York). Blood lactate, β-hydroxybutyrate, and triglycerides were measured by enzymatic methods and insulin by a double-antibody radioimmunoassay. All the assay methods have been previously reported from this laboratory.[15]

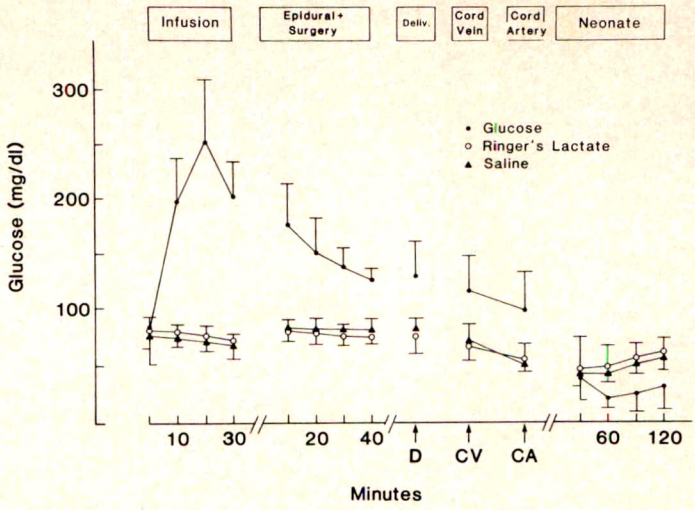


Fig. 1. Changes in plasma glucose concentration in the mother and infant after intravenous fluid administration. *D* = At delivery; *CV* = cord vein; *CA* = cord artery. Data are presented as mean ± SD.

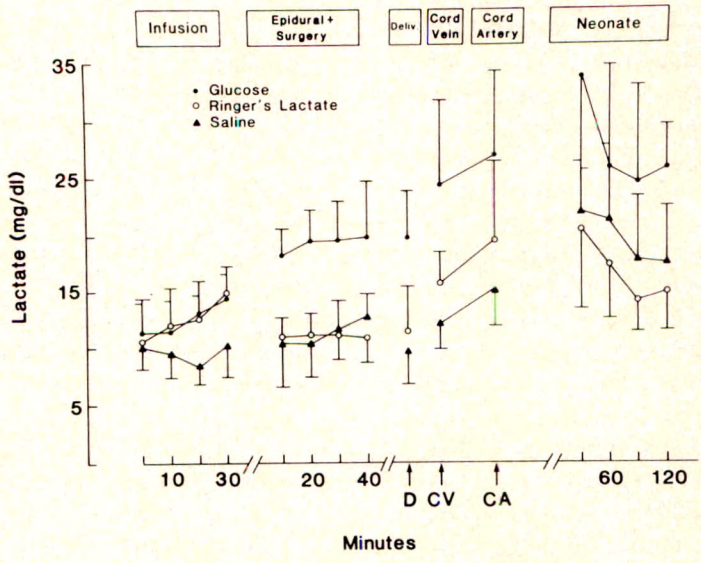


Fig. 2. Changes in blood lactate concentration in the mother and infant after intravenous administration. For abbreviations see legend to Fig. 1.

All data are presented as mean ± SD. Group comparisons were done with Student's *t* test. A p value of <0.05 was considered statistically significant.

Results

All patients were at term and did not have any underlying medical problems. As shown in Table I, there were no significant differences among the three groups in their antenatal characteristics. The neonatal birth weight and gestational age at birth were also similar between the groups. The neonates were all term sized, appropriate for gestational age, and had 5-minute Apgar scores >7. One neonate in group 1 had meconium-stained amniotic fluid below the vocal cords and had a 1-minute Apgar score of 6. The 5-minute Apgar score in this neonate was 8.

Plasma glucose. The changes in plasma glucose concentration in the three groups are displayed in Fig. 1. The initial maternal plasma glucose concentration was similar in the three groups. Acute intravenous glucose (50 gm during 30 minutes) administration in group 1 caused a rapid increase in plasma glucose concentration, reaching a maximum of 225 ± 66 mg/dl at 20 minutes followed by a continuous gradual decline. At delivery, the maternal plasma glucose level in group 1 was still significantly elevated (134 ± 37

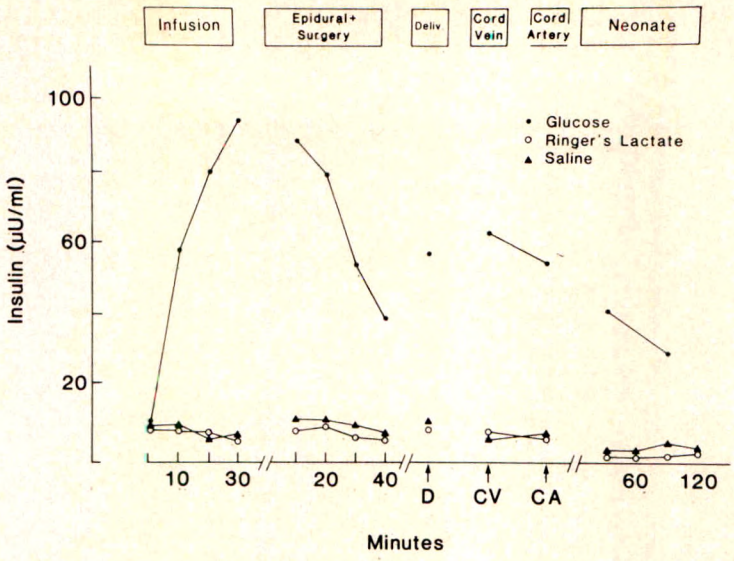


Fig. 3. Changes in plasma insulin concentration in the mother and infant after intravenous fluid administration. For abbreviations see legend to Fig. 1.

mg/dl). In contrast, acute maternal hydration with Ringer's lactate or with normal saline solution caused a small but insignificant decrease in plasma glucose concentration. The glucose concentration remained stable thereafter, so that at delivery the maternal plasma glucose concentration was 83 ± 16 mg/dl in group 2 and 76 ± 9 mg/dl in group 3.

In all three groups, the umbilical cord venous and arterial glucose concentrations were lower than the simultaneously obtained maternal peripheral venous glucose levels at delivery. However, in the group administered glucose, the umbilical cord venous and arterial glucose concentrations were significantly elevated when compared with those of groups 2 and 3 ($p < 0.001$).

Even though the glucose concentration in the umbilical cord vein and artery was higher in group 1 than in groups 2 and 3, the plasma glucose concentration in the newborn infants at 30 minutes was similar in all three groups. The plasma glucose concentration in group 1 decreased during the next 30 minutes before stabilizing during the next hour. By 2 hours after birth, the neonatal plasma glucose concentration remained significantly lower in group 1 than in groups 2 and 3 ($p < 0.01$). In contrast, the plasma glucose concentration in the newborn infants in groups 2 and 3 stabilized between 44 and 62 mg/dl for the next 90 minutes.

Four of 12 neonates in group 1 and two of 11 neonates in group 2 developed asymptomatic hypoglycemia (blood glucose value of <25 mg/dl, Dextrostix, Ames Co., Elkhart, Indiana). No infants in group 3 developed hypoglycemia. One infant in group 1 developed tachypnea. At this time, the plasma glucose concentration was 18 mg/dl.

Blood lactate. Changes in maternal, umbilical cord, and neonatal blood lactate concentrations are shown in Fig. 2. Initial maternal blood lactate concentrations in groups 1, 2, and 3 were 12 ± 3, 11 ± 3, and 10 ± 2 mg/dl, respectively. In groups 1 and 2, the maternal blood lactate concentration was significantly higher at the end of the intravenous infusion when compared with the respective initial blood lactate concentrations and also when compared with the value in the group receiving normal saline solution (group 3) ($p < 0.01$). However, by the time the epidural anesthesia was induced and surgery was begun, the blood lactate concentration in group 2 was similar to that of group 3. The maternal blood lactate concentration in the glucose infusion group remained significantly elevated throughout surgery and delivery when compared with the initial blood lactate concentration and also when compared with values in groups 2 and 3.

The umbilical cord venous and arterial blood lactate concentrations were higher than the maternal venous blood lactate concentrations in all three groups. The glucose infusion in group 1 caused a greater increase in the blood lactate concentration in the umbilical cord vein ($p < 0.001$) and artery ($p < 0.002$) when compared with that of groups 2 and 3. In addition, the blood lactate concentration was higher in the umbilical artery than in the vein in all three groups. The blood lactate concentration in the neonates at 30 minutes after birth was significantly higher in group 1 than in groups 2 and 3 ($p < 0.004$), but by 90 minutes the lactate concentration in the three groups of neonates was not statistically different.

Plasma insulin. The initial maternal plasma insulin concentrations in groups 1, 2, and 3 were 8.3 ±

Table II. Acid-base status at delivery

	Group 1: Glucose (N = 12)	*Group 2: Ringer's lactate (N = 11)*	*Group 3: Normal saline (N = 9)*
Maternal vein at delivery			
pH	7.36 ± 0.04	7.36 ± 0.12	7.37 ± 0.05
P_{CO_2}	35.1 ± 5.3	33.7 ± 4.7	34.8 ± 6.9
P_{O_2}	61.7 ± 41.1	68.7 ± 43.6	65.1 ± 31.0
Base deficit	−4.9 ± 2.0	−4.7 ± 6.3	−3.7 ± 2.3
Cord vein			
pH	7.31 ± 0.04*	7.35 ± 0.07	7.34 ± 0.04
P_{CO_2}	41.8 ± 4.8	40.6 ± 7.0	39.8 ± 4.8
P_{O_2}	31.4 ± 8.9	30.5 ± 14.9	31.1 ± 4.6
Base deficit	−4.7 ± 2.5	−2.7 ± 1.9	−3.7 ± 3.6
Cord artery			
pH	7.21 ± 0.06*	7.27 ± 0.12	7.27 ± 0.06
P_{CO_2}	57.7 ± 5.6*	49.2 ± 8.4	50.8 ± 6.0
P_{O_2}	14.0 ± 3.3	17.1 ± 5.4	15.0 ± 3.6
Base deficit	−5.1 ± 3.0	−3.1 ± 2.7	−4.3 ± 3.4
Oxygen saturation	11.8 ± 7.0*	22.7 ± 12.3	17.6 ± 6.7

Values are mean ± SD.
*$p < 0.05$ (group 1 compared with groups 2 and 3).

6.2, 10.5 ± 5.5, and 12.6 ± 9.0 μU/ml, respectively (Fig. 3). The glucose infusion in group 1 caused a short-term rise in the insulin concentration so that at 20 minutes the plasma insulin concentration was 80.7 ± 44.8 μU/ml ($p < 0.0001$). At delivery, the maternal plasma insulin concentration in group 1 remained significantly elevated at 58.9 ± 38.4 μU/ml ($p < 0.002$) with the plasma insulin concentrations in groups 2 and 3 remaining unchanged. The insulin concentration in the umbilical cord vein (65.9 ± 67.9 μU/ml) and artery (55.6 ± 66.5 μU/ml) at delivery in group 1 was also significantly elevated when compared with the other two groups ($p < 0.02$). The insulin concentrations could not be measured in all the neonates at 30, 60, 90, and 120 minutes because of limited blood sample size. However, the neonatal insulin concentrations in groups 2 and 3 remained <3.5 μU/ml while in the neonates of group 1 higher insulin concentrations were maintained at 30 and 90 minutes after birth.

Acid-base status. The maternal venous pH before the infusion was similar in the three groups. By the end of the infusion, the maternal pH in group 1 (7.36 ± 0.03) was significantly lower than the pH in group 2 (7.38 ± 0.2) and group 3 (7.39 ± 0.02) ($p < 0.05$). As shown in Table II, the maternal pH at delivery was similar between the groups. However, the umbilical arterial pH in group 1 (7.21 ± 0.06) was significantly lower than the umbilical arterial pH in group 2 (7.27 ± 0.12) and group 3 (7.27 ± 0.06) ($p < 0.05$). The umbilical venous blood pH in group 1 (7.31 ± 0.04) was also significantly lower than the umbilical cord vein pH in group 2 (7.35 ± 0.07) and group 3 (7.34 ± 0.04) ($p < 0.05$). In the neonatal period, there was a tendency for lower venous pH values in the neonates from group 1. However, no conclusions can be made as complete studies were done in only eight neonates.

Additional acid-base results in the maternal vein at delivery and the umbilical cord vein and artery of the three groups are shown in Table II. There was a significantly higher umbilical cord artery P_{CO_2} in the glucose-infused group. The umbilical arterial hemoglobin oxygen saturation in the glucose group (11.8 ± 7.0) was significantly lower than that of the Ringer's lactate (22.7 ± 12.3) or normal saline solution (17.6 ± 6.7) groups ($p < 0.05$).

β-Hydroxybutyrate and triglycerides. As shown in Table III, acute glucose infusion in group 1 was associated with a significant decrease in the concentration of β-hydroxybutyrate in the maternal blood at delivery ($p < 0.001$). In addition, the umbilical venous ($p < 0.003$) and arterial ($p < 0.04$) concentrations of β-hydroxybutyrate were lower in group 1 than in groups 2 and 3. The concentration of β-hydroxybutyrate in the maternal plasma tended to rise in the Ringer's lactate and normal saline solution groups. The triglyceride levels in the three groups demonstrated wide variations with large standard deviations within each group. No significant differences in the mean triglyceride levels between the groups were identified.

Serum electrolytes, carbon dioxide, and blood urea nitrogen. The serum electrolytes, carbon dioxide, and blood urea nitrogen were similar in the three groups. Acute glucose infusion significantly decreased the serum sodium level from 137 ± 3 to 131 ± 3 mEq/L ($p < 0.01$). No significant change in other electrolytes, carbon dioxide, or blood urea nitrogen was seen in any of the groups.

Table III. Changes in β-hydroxybutyrate and triglycerides in the three groups

	β-Hydroxybutyrate (mmol/L)			*Triglycerides (mg/dl)*		
	Group 1: Glucose	*Group 2: Ringer's lactate*	*Group 3: Normal saline*	*Group 1: Glucose*	*Group 2: Ringer's lactate*	*Group 3: Normal saline*
Mother						
Preinfusion	0.23 ± 0.26	0.21 ± 0.09	0.24 ± 0.15	209 ± 112	221 ± 91	233 ± 112
Delivery	0.11 ± 0.07*	0.26 ± 0.15	0.33 ± 0.16	183 ± 95	214 ± 83	229 ± 91
Umbilical cord						
Vein	0.09 ± 0.04†	0.12 ± 0.04	0.17 ± 0.07	15 ± 9	18 ± 12	13 ± 9
Artery	0.07 ± 0.03‡	0.12 ± 0.06	0.10 ± 0.02	12 ± 5	16 ± 9	12 ± 5
Neonate						
30 min	0.09 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	23 ± 5	16 ± 11	20 ± 5
60 min	0.08 ± 0.02	0.07 ± 0.03	0.06 ± 0.03	24 ± 8	22 ± 8	20 ± 8

Values are mean ± SD.
*$p < 0.001$, compared with groups 2 and 3.
†$p < 0.003$, compared with groups 2 and 3.
‡$p < 0.04$, compared with groups 2 and 3.

Table IV. Influence of maternal hypotension on pH, glucose, and lactate at delivery

	Hypotension (N = 9)	*No hypotension (N = 23)*	*Significance*
Maternal vein at delivery			
pH	7.31 ± 0.12	7.38 ± 0.04	$p < 0.05$
Glucose	126 ± 49	90 ± 24	$p < 0.01$
Lactate	19.1 ± 5.3	12.6 ± 5.0	$p < 0.01$
Umbilical cord vein			
pH	7.30 ± 0.04	7.34 ± 0.05	$p < 0.05$
Glucose	111 ± 45	78 ± 22	$p < 0.01$
Lactate	23.9 ± 9.8	15.9 ± 6.0	$p < 0.01$
Umbilical cord artery			
pH	7.19 ± 0.11	7.27 ± 0.06	$p < 0.02$
Glucose	95 ± 42	62 ± 20	$p < 0.01$
Lactate	26.3 ± 9.3	18.8 ± 7.1	$p < 0.05$

Values are mean ± SD.

Maternal hypotension. The influence of maternal hypotension on pH, plasma glucose, and blood lactate are shown in Table IV. Nine patients (28%) developed hypotension: six in group 1, two in group 2, and one in group 3. In comparison with the group that remained normotensive, the hypotensive group of patients had a significantly lower maternal vein pH at delivery as well as a lower pH in the umbilical cord vein and artery. Because the hypotensive patients included a greater proportion from the glucose group, this group also had elevated plasma glucose and blood lactate levels in the maternal vein at delivery and in the umbilical cord. In addition, all of these patients had received ephedrine for the correction of hypotension.

Because of these differences in plasma glucose and blood lactate in the hypotensive group, the influence of maternal hypotension was examined within group 1 alone. When the six hypotensive patients were compared with six normotensive patients in group 1, there were no significant differences in the pH, plasma glucose level, or blood lactate level. The glucose concentration in the maternal venous blood at delivery tended to be higher, although not significantly so, in the hypotensive group, possibly because of ephedrine administration.

Uterine incision–to–delivery interval. In order to examine the effects of a prolonged uterine incision–to–delivery interval, patients with a uterine incision–delivery interval >180 seconds were compared with patients with a uterine incision–delivery interval of <180 seconds. A prolonged uterine incision–to–delivery interval did not significantly alter any of the parameters measured.

Comment

The purpose of this study was to evaluate the maternal and fetal metabolic effects of three commonly used intravenous fluids during delivery. The results

demonstrate that acute glucose infusion before delivery resulted in maternal hyperglycemia, hyperinsulinemia, and an increase in blood lactate. These maternal perturbations were associated with fetal hyperglycemia and hyperinsulinemia, higher fetal blood lactate levels, and lower fetal arterial pH.

The key finding of this study was the lower fetal arterial pH in the glucose-infused group. This increase in blood hydrogen ion concentration was accompanied by a significant rise in blood lactate, an increase in carbon dioxide, and a decreased fetal hemoglobin oxygen saturation. An explanation for this lactic acidosis may be obtained from animal data. In chronically catheterized ewes, direct glucose infusion to the fetus can induce an acceleration of the fetal metabolic rate and fetal oxygen consumption.[7] The increase in fetal oxygen consumption has been speculated to be responsible for the decrease in fetal arterial oxygen content. Robillard et al.[5] have shown that chronic fetal hyperglycemia in sheep induces a similar state of fetal arterial hypoxia, metabolic acidosis, and fetal death. Direct infusion of insulin to the fetus caused an increased glucose uptake by the fetus and a decreased fetal arterial oxygen content.[8, 9] Crandell et al.[16] showed that in chronically catheterized ewes hyperglycemia resulted not only in fetal hypoxemia and acidemia but also in a redistribution of the fetal combined ventricular output and a decrease in umbilical blood flow to the placenta. Although there was no significant change in oxygen delivery to the fetal heart, brain, or kidneys in this study, there was a marked decrease in fetal arterial oxygen saturation. On the basis of these studies in animals, one may speculate that acute maternal glucose infusion in human pregnancy results in an increased metabolic rate of the fetus. Glucose consumption and lactic acid production are increased. Fetal oxygen consumption is also increased and results in a decrease in fetal oxygen content and subsequent fetal hypoxemia. Fetal hypoxemia leads to a decrease in pH and, if severe, lactic acidosis. Redistribution of fetal cardiac output may be a compensatory adaptation to these metabolic changes.

This study also examined other metabolic consequences of acute glucose infusion. Acute maternal hyperglycemia persisted more than 40 minutes after the infusion so that, by delivery, the maternal glucose remained elevated and the concentration of blood lactate had doubled. The umbilical cord vein and artery blood lactate concentrations remained twice as high as those of the control group. In contrast, the group that received the Ringer's lactate (28 mEq/L of lactate) did not demonstrate this altered metabolic lactic acidosis.

Other perinatal factors, such as maternal position, maternal hypotension, and surgical time intervals at the time of cesarean section, were examined since these factors are known to influence fetal acid-base status at delivery.[9-13] All patients in this study had left lateral uterine displacement to avoid the effects of aortocaval compression. Maternal hypotension was rapidly corrected and did not influence neonatal acid-base status when controlled for glucose concentration. In this study, a prolonged skin-to-delivery interval did not influence the neonatal acid-base balance. Similar results have been reported with spinal anesthesia in the absence of hypotension.[11] The uterine incision–to–delivery interval also did not appear to influence the pH although there were only a few cases with a prolonged interval of >3 minutes. Therefore, by controlling for these confounding variables, the alterations in substrates and lower pH were attributed to the metabolic effects of acute glucose infusion alone.

In summary, short-term maternal administration of glucose before delivery is associated with fetal hyperglycemia, hyperinsulinemia, an increase in lactic acid, and a significant decrease in pH. Nevertheless, no obvious detrimental effects were observed on the mother, fetus, or neonate in this study. However, these metabolic changes occurred in normal patients at term without any signs of fetal distress or acidosis during the infusion, epidural anesthesia, or surgery. Since fetal distress and acidosis may occur during labor and delivery, acute glucose infusion in this clinical setting may exaggerate the metabolic acidosis associated with fetal distress. When intravenous fluids are necessary in obstetrics, either for hydration or administering medications, large amounts of glucose should be avoided.

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The use of fetal neuromuscular blockade during intrauterine procedures

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Advances in fetal therapy have led to the utilization of such techniques as intravascular transfusion of the Rh-affected fetus, bladder shunt placement in the fetus with obstructive uropathy, and percutaneous umbilical blood sampling. Fetal movement makes these procedures technically more difficult while increasing the risk of fetal injury. However, maternal sedation rarely results in adequate suppression of fetal activity. Thus we tested the sedative effects of intramuscular *d*-tubocurarine (3 or 1.5 mg/kg) or pancuronium bromide (0.3 mg/kg) injected into the fetal gluteal region under ultrasound guidance in conjunction with 70 invasive in utero procedures. Short-term paralysis of the fetus was induced in all cases. No deleterious effects of this technique were noted on initial examination of the neonates. Neuromuscular blockade was found to be a very useful adjunct to both diagnostic and therapeutic procedures involving the fetus. (Am J Obstet Gynecol 1987;157:874-9.)

Key words: Fetus, *d*-tubocurarine, pancuronium bromide, fetal paralysis

In utero fetal therapy began in 1963 with the report by Liley[1] of the first successful intraperitoneal transfusion for severe rhesus alloimmunization. Later, Bang et al.[2] used ultrasound-guided needle placement to perform fetal intravascular transfusions. The fetus has now become the target of numerous invasive diagnostic and therapeutic maneuvers that range from percutaneous umbilical blood sampling[3] to placement of intravesical shunts.[4] Fetal movement makes these procedures technically more difficult and may increase the likelihood of trauma to the umbilical vessels or injury to the fetus. Efforts to suppress fetal activity by maternal sedation often meet with only partial success. Using ja combination of meperidine, prochlorperazine, and diazepam, Berkowitz et al.[5] reported that the fetus dislodged the needle in 11% of the intravascular transfusions in their series. Ch. de Crespigney et al.[6] were the first to use intramuscular *d*-tubocurarine to achieve fetal paralysis before intravascular transfusion. Our purpose is to describe our experience with fetal neuromuscular blockade used in conjunction with invasive in utero procedures.

Material and methods

Patients. Between June 1985 and March 1987, neuromuscular blocking agents were used in conjunction with 70 procedures involving 31 fetuses at the Prenatal Diagnostic Center, Baylor College of Medicine, Houston, Texas. In 13 cases two separate procedures were undertaken while the fetus was paralyzed (fetal blood sampling followed by either intraperitoneal transfusion or combined intravascular and intraperitoneal transfusions). The types of procedures and gestational ages when fetal paralysis was used are shown in Table I. Fetal paralysis was used five times in two fetuses, four times in two fetuses, three times in three fetuses, twice in six fetuses, and once in 18 fetuses.

Method of procedure. Fetal weight was calculated by use of the ultrasound parameters of biparietal diameter and abdominal circumference.[7] If ascites were present, the 50th percentile weight for the corresponding menstrual age was used. In the first 44 procedures, an undiluted concentration of 3 mg/ml of *d*-tubocurarine was used to administer a dose of 3 mg/kg estimated fetal weight (group 1). In four subsequent cases the dose was reduced to 1.5 mg/kg (group 2). In the last 12 cases, an undiluted concentration of 1 mg/ml of pancuronium bromide was used to administer 0.3 mg/kg of the estimated fetal weight (group 3). The fetal lie was then assessed with ultrasound. If the fetus was felt to be in a poor position for a particular procedure (e.g., fetal spine anterior for an intraperitoneal transfusion), an attempt was made to improve the position by external manipulation before the institution of fetal paralysis. The fetal femur closest to the anterior uterine wall was localized with real-time ultrasound. A 25-gauge spinal needle was then guided into the fetal gluteal region and the neuromuscular agent was injected. Correct placement was confirmed by the appearance of an echo-

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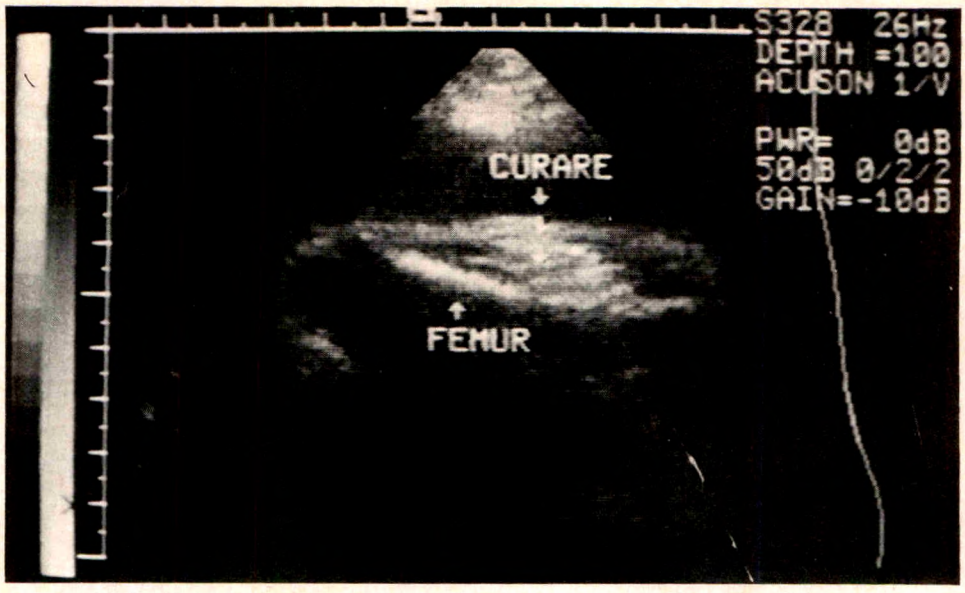


Fig. 1. Fetal gluteal region after intramuscular injection of *d*-tubocurarine.

Table I. Types of procedures performed in conjunction with fetal neuromuscular blockade

Procedure	*No. of procedures*	*Gestational age (wk)*		
		Mean	*Minimum*	*Maximum*
Percutaneous umbilical blood sampling	17	24.6	17.6	34.7
Intrauterine transfusion				
Intraperitoneal	31	28.8	21.0	35.6
Intravenous	17	28.9	23.3	37.6
Fetal skin biopsy	1	20.0	—	—
Paracentesis	1	25.4	—	—
Thoracentesis	1	34.0	—	—
Placement of intraperitoneal shunt	1	26.0	—	—
Cardiac puncture	1	17.6	—	—

genic area in the fetal thigh (Figs. 1 and 2). Fetal activity was monitored with continuous real-time ultrasound until no limb movement could be detected. The latency period of a particular drug or dosage was defined as the interval from its injection to the cessation of fetal movement. Most patients were discharged before spontaneous reversal of the neuromuscular blockade. Patients were asked to record the time when they first perceived fetal movement. The duration of action of a particular neuromuscular drug or dosage was defined as the time interval between injection and maternal perception of the return of fetal movement.

Results

General experience. Fetal paralysis was achieved in all fetuses. A second injection of an agent was required in 10 cases, probably because of poor placement of the drug. In these cases, the lack of an echogenic area in the fetal gluteal region or the lack of resistance during injection was observed, indicating intra-amniotic placement of the drug.

The mean latency period for the various treatment groups was as follows: Group 1, 3.2 minutes (range 1 to 15 minutes); group 2, 5.1 minutes (range 4 to 6.5 minutes); and group 3, 3.8 minutes (range 1.5 to 16 minutes). The last eight patients in group 1 and all patients in groups 2 and 3 were asked to record the time when they first perceived the return of fetal movement. The mean duration of *d*-tubocurarine action in group 1 was 9.0 hours (range 4.7 to 16 hours) and was 3.8 hours (range 2 to 4.8 hours) in group 2. The mean duration of action of pancuronium bromide (group 3) was 6.8 hours (range 2 to 11.5 hours).

The mean interval between fetal paralysis and delivery was 41 days (range 5 hours to 147 days). Two pregnancies were electively terminated after chromosomal abnormalities were confirmed by fetal blood sampling. One in utero death occurred in a severely hydropic

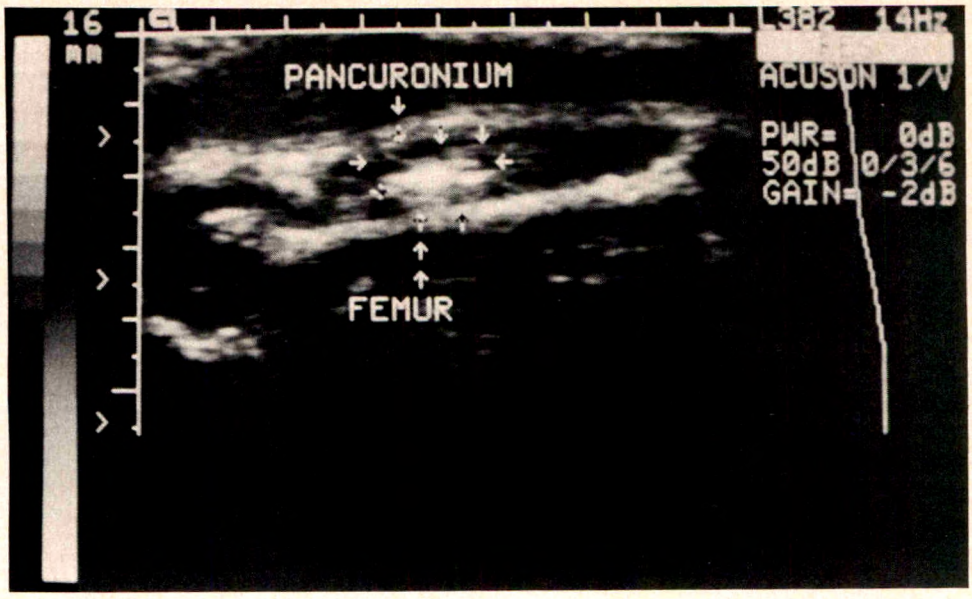


Fig. 2. Fetal gluteal region after injection of pancuronium bromide.

fetus at 22.4 weeks' gestation after intrauterine transfusion for rhesus alloimmunization. Two infants died in the immediate neonatal period. One of these infants was delivered at 28 weeks' gestation because of chorioamnionitis that developed after an intrauterine transfusion for rhesus disease. The infant died secondary to severe hyaline membrane disease. The second infant was delivered by emergency cesarean section at 33 weeks' gestation after abruptio placentae and died as a result of the asphyxial insult. Of the remaining 26 fetuses, three are currently undelivered. The remaining 23 infants were live born and showed no evidence of ill effects secondary to in utero fetal paralysis.

Special cases

Case 1. A 21-year-old woman, para 1-0-0-1, was scheduled to undergo a second intravascular transfusion at 37 weeks' gestation due to rhesus alloimmunization. Amniotic fluid analysis several days before the procedure revealed lack of fetal pulmonary maturity. Estimated fetal weight was 3000 gm. The calculated dose of *d*-tubocurarine for fetal neuromuscular blockade was 9 mg (3 ml of a 3 mg/ml concentration). This volume was felt to be too large for intramuscular injection into the fetal gluteal region. A 0.3 mg/kg dose of pancuronium bromide (total volume 0.9 ml) was thus used. Cessation of fetal movement occurred after 2 minutes and lasted 2 hours. A successful intravascular transfusion was performed without incident.

Case 2. A 25-year-old woman, para 2-0-0-2, underwent an initial intravascular transfusion for fetal hydrops secondary to Kell alloimmunization. Fetal neuromuscular blockade was not instituted because of the large anterior placenta that precluded easy access to the fetal thigh and the relative lack of fetal movement. The procedure needle was guided through the anterior placenta into the umbilical cord insertion without difficulty. Several minutes after the start of the transfusion, the fetus was noted to become very active. Fetal movement against the cord insertion site resulted in displacement of the transfusion needle through the vessel wall. Active bleeding into the amniotic cavity was seen and the needle was immediately withdrawn. The bleeding from the puncture site subsided without evidence of fetal bradycardia.

Case 3. A 31-year-old woman, para 0-0-3-0, was noted to have evidence of fetal hydrops at 34 weeks' gestation. A large right-sided pleural effusion with resulting displacement of the mediastinum against the left chest wall was present. Amniotic fluid analysis revealed borderline pulmonary maturity study results. After the administration of steroids, cesarean section was to be undertaken. After consultation with the neonatology service, a decision was made to perform in utero thoracentesis before delivery to minimize resuscitative efforts at delivery. Pancuronium bromide, 0.3 mg/kg, was administered into the fetal gluteal region. Fetal neuromuscular blockade was complete 3 minutes later. A successful thoracentesis was undertaken, and 120 ml of fluid was removed. A cell count on the fluid revealed a total white blood cell count of 1100 cells/mm^3, consisting of 90% lymphocytes. This finding was felt to be consistent with a congenital chylothorax. After 4 hours, ultrasound revealed vigorous fetal limb movement and cesarean section was performed. The neonate was given 1- and 5-minute Apgar

scores of 9 and 9 and exhibited spontaneous respirations and limb movement at birth. The early neonatal course was complicated by hyaline membrane disease. The infant began oral feedings with a medium-chain triglyceride formula on the seventh day of life. Serial chest radiographs demonstrated resolution of the right-sided pleural effusion. Discharge occurred on the nineteenth day of life.

Comment

Tubocurarine was the first agent used for fetal neuromuscular blockade.[6] Ch. de Crespigney et al. arbitrarily chose a dose of 3 mg/kg, which represents a tenfold increase in the usual recommended intravenous dose for neonatal anesthesia.[8] In the original report the drug was administered and then followed 30 minutes later by intravascular transfusion.[6] The fetus was not monitored by ultrasound during the interim; therefore the latency period of the drug was not known. Our initial experience with the drug indicated that its onset of action was rapid (mean time 3.3 minutes). When we used the 3 mg/kg dose described by Ch. de Crespigney et al., we noted that the fetus remained paralyzed for a prolonged period (mean 9 hours). The duration of action of the drug was felt to be excessive. In an effort to reduce the length of fetal paralysis, we used a 50% reduction in the dose of *d*-tubocurarine. This resulted in a slight prolongation of the mean latency period (from 3.2 to 5.1 minutes) before the onset of fetal neuromuscular blockade. More importantly, the duration of fetal neuromuscular blockade was reduced by more than half (mean 3.8 hours).

When faced with a need for fetal paralysis in the first case described, we decided to select an alternative agent to *d*-tubocurarine. The pharmacology of the various neuromuscular agents was considered in the selection of a new agent for fetal neuromuscular blockade. Three agents are currently in popular use: vecuronium, atracurium, and pancuronium bromide. Vecuronium is excreted primarily by the liver and would be suboptimal for use in the Rh-affected fetus, because the liver may be involved in extrahematopoietic red cell production.[9] Atracurium is metabolized in the vascular space through Hoffman degradation and ester hydrolysis.[10] Although these two agents are reported to have minimal cardiovascular effects, their short half-life was felt to be a disadvantage when extended in utero procedures are planned.[10] Renal excretion serves as the primary mode of elimination for pancuronium bromide,[10] while hepatic metabolism with excretion into the biliary tree is an alternative route of excretion.[10] Pancuronium bromide would therefore represent an agent with little potential for drug-related hepatic toxicity. Considerable experience has been gained with this drug in neonates. It is noted to be approximately five times more potent than *d*-tubocurarine, allowing its use in very small volumes.[11] We calculated a dose of 0.3 mg/kg based on the 1.5 mg/kg dose of *d*-tubocurarine used in our second group of fetuses. Pancuronium bromide was noted to have a rapid onset of action (similar to 3 mg/kg of *d*-tubocurarine), with a duration of fetal neuromuscular blockade intermediate between the 1.5 and 3 mg/kg *d*-tubocurarine doses. This length of fetal paralysis was felt to be adequate to perform even the most difficult intravascular exchange transfusion. Recently, Seeds et al.[12] have reported the use of intramuscular pancuronium bromide in six cases of fetal neuromuscular blockade. In all cases cessation of fetal movement had occurred by 1 minute after injection, with return of movement by 2 hours. These authors did not calculate the dose of pancuronium bromide based on fetal weight but instead used a standard dose of 0.5 mg. The oldest fetus in their series was 31 weeks' gestation and would have a fiftieth percentile estimated fetal weight of 1630 gm.[13] Based on a dose of 0.3 mg/kg, we would have administered 0.49 mg of pancuronium bromide as compared with the 0.5 used by Seeds et al. Calculation of a dose based on fetal weight would seem to follow sound pharmacologic principles and would allow the use of doses <0.5 mg for fetal neuromuscular blockade at earlier gestational ages.

Although neuromuscular blocking agents are usually administered to the neonate by the intravenous route, we chose to follow the protocol of Ch. de Crespigney et al.[6] and use the intramuscular route of administration. The intramuscular route offers several advantages over the intravenous approach: (1) fetal neuromuscular blockade can be induced before umbilical vessel puncture for transfusions or percutaneous umbilical blood sampling, enabling easier placement of the needle after the cessation of fetal movement; and (2) fetal neuromuscular blockage effected in this manner allows for fetal paralysis in conjunction with such procedures as fetal skin biopsy, in which access to the fetal vascular space is not part of the usual procedure. Disadvantages of intramuscular injection in the fetus include the need for a second amniotic puncture and the risk of possible injury to structures in the fetal thigh. Seeds et al.[12] used a 22-gauge needle for the intramuscular injection of pancuronium bromide. We have found a 25-gauge needle to be more than adequate for this technique and feel that the smaller needle should theoretically reduce the incidence of complications. To date, no evidence of gluteal hematomas or nerve injury has been noted in any of the neonates in our series of fetal neuromuscular blockade.

Indications for the use of fetal paralysis are varied. Umbilical vessels near the placental cord insertion are

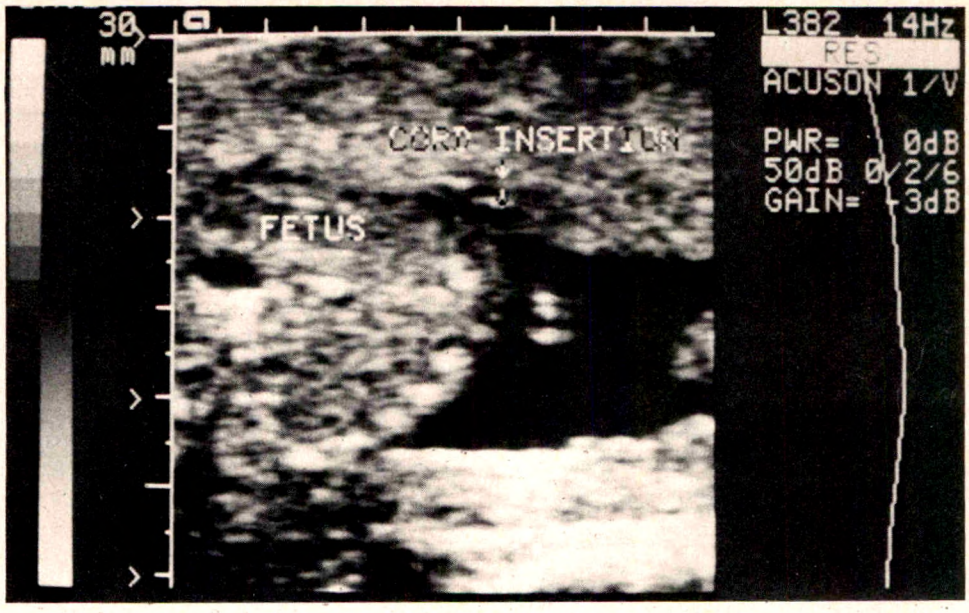


Fig. 3. Fetus obscuring the cord insertion of an anterior placenta.

probably the most commonly used sites for intravascular fetal transfusion.[5, 14] In cases of posterior placentation, fetal movement may result in dislodgement of the needle once it has been placed. In addition, the fetal lie may obscure the posterior cord insertion, making use of this site technically impossible. Attempts to puncture the vessels in a floating loop of umbilical cord are equally difficult. The intrahepatic portion of the umbilical vein or the cord vessels at the fetal umbilicus have been used by some investigators to circumvent these problems.[2, 5, 6] If the hydropic fetus is moribund, these sites may be relatively safe due to the lack of fetal activity. Nonetheless, fetal movement may increase in response to the acute correction of anemia, placing the fetus at risk for vessel trauma. The use of neuromuscular blockade in the fetus would therefore appear to be very advantageous when intravascular transfusion is undertaken with a posterior placentation.

If an anterior placentation is present, puncture of the umbilical vessels is usually technically easier. In this situation, one may question the need for fetal paralysis. Although the needle will not usually enter the amniotic cavity if the cord root is approached on end, occasionally the needle may puncture the cord a short distance from the actual insertion, resulting in a short segment of needle in the amniotic cavity. Fetal movement near the cord insertion may result in difficult visualization of the umbilical vessels (Fig. 3). Our second case illustrates that fetal movement against the cord insertion site can result in displacement of the needle through the vessel wall, with the potential for severe trauma to the umbilical vessels. For this reason, we would advocate the use of fetal paralysis for all intravascular transfusions even if the placenta is located anteriorly.

Fetal movement during intraperitoneal transfusion may place the fetus at risk for internal visceral injury. Bowman and Manning[15] have advocated the placement of intraperitoneal catheters to decrease the length of time the needle remains in the fetus. Fetal paralysis enables the proper placement of the needle followed by unhurried transfusion of blood, negating the need for catheters.

Percutaneous umbilical fetal blood sampling can be technically difficult during one's initial experience. Although we no longer use fetal neuromuscular blockade during percutaneous umbilical blood sampling, we found it to be very useful in our earlier cases. After one masters the technique of umbilical cord sampling, fetal paralysis may no longer be warranted. Occasionally the very active fetus will make puncture of a posterior cord insertion technically difficult. In these cases, the use of a neuromuscular agent would substantially increase the ease of the procedure.

Finally, we have found fetal paralysis to be beneficial during such procedures as paracentesis, thoracentesis, skin biopsy, and shunt placement.

Several minor disadvantages of fetal neuromuscular blockade merit discussion. The latency period after injection of the drug is usually several minutes. During this period the fetus may assume a position that would make a particular procedure very difficult. Manipulation of the fetus can be attempted, but we advocate that this be done cautiously. The lack of muscle tone secondary to fetal paralysis could potentially place the fe-

tus at risk for iatrogenic skeletal injury. Changes in fetal posture may also occur after neuromuscular blockade of the fetus. On several occasions, marked flexion of the fetal spine and extremities (a "balled-up" position) made access for intraperitoneal transfusion difficult. Another potential disadvantage to fetal neuromuscular blockade would occur if emergency delivery must be effected secondary to fetal distress during a procedure. A paralyzed infant would be delivered. Atropine and neostigmine could be administered in an attempt to reverse the fetal paralysis; however, these agents are not likely to be effective if spontaneous resolution of fetal paralysis is not present.[16] Our experience has shown that the duration of fetal neuromuscular blockade after intramuscular injection of *d*-tubocurarine or pancuronium bromide indicates that endotracheal intubation and assisted ventilation for several hours after birth would be necessary. Our third case illustrates that fetal neuromuscular blockade can, however, be used just before scheduled delivery if sufficient time is allowed for spontaneous recovery.

Although we have found fetal neuromuscular blockade to be an invaluable adjunct to invasive in utero procedures, the long-term effects of neuromuscular blocking agents in the fetus have not been studied. Neuromuscular blockade of the fetus is a short-lived effect and would not be expected to have subsequent detrimental consequences. Prospective evaluation of these infants is ongoing at our institution.

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Fetal assessment based on fetal biophysical profile scoring: Experience in 19,221 referred high-risk pregnancies

II. An analysis of false-negative fetal deaths

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The incidence of false-negative fetal death, which is defined as stillbirth unrelated to major anomaly or alloimmunization occurring after a last normal fetal biophysical score, was determined in 19,221 referred high-risk pregnancies. The calculated rate of fetal death after a last normal test was 0.726/1000 (14 deaths), which remained relatively constant despite a progressive increase in tests and patients studied. We conclude that a normal fetal biophysical profile score confers a high probability of perinatal survival. (AM J OBSTET GYNECOL 1987;157:880-4.)

Key words: Fetal biophysical profile, stillbirth, false-negative test

Fetal biophysical profile scoring is a method of antepartum fetal risk assessment based on a dynamic ultrasound survey of biophysical variables.[1] According to the experience reported from our center and other independent clinical laboratories, the method when applied to a high-risk obstetric population yields a reduction in the perinatal mortality rate.[2-4]

One unique characteristic of the testing method is the high proportion of normal test results, which has been reported as 97.5% among 30,411 tests.[2, 3] Paradoxically, this high rate of normal test results could be the result of either high- or low-negative predictive accuracy of the testing method. The prevalence of perinatal disease, as defined by the sharpest end point, perinatal death, is low even among the selected high-risk population; conversely, the expectation of live birth is high. A testing method of high negative predictive accuracy applied to this population would therefore yield a high proportion of normal results, mirroring the population expectation. Conversely, a test of lesser negative predictive accuracy by which the compromised fetus may be assigned a normal test result could also yield the same high proportion of normal test results. Resolution of this issue as it pertains to fetal biophysical profile scoring is of considerable clinical importance. Accordingly, we have reviewed our experience with this testing method in 19,221 referred high-risk pregnancies. The purpose of this retrospective study was twofold: to catalog all false-negative fetal deaths by number and associated etiologic factors and to calculate the probability of a fetal death within 1 week of a last normal fetal biophysical profile score.

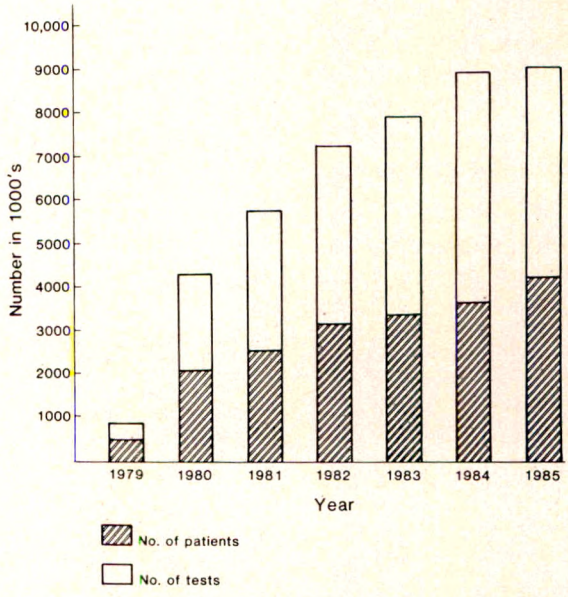


Fig. 1. The annual distribution of the number of patients referred for fetal biophysical profile scoring and the number of tests performed.

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Table I. Biophysical profile scoring: Management protocol

BPS score	*Interpretation*	*Recommended management*
10	Normal nonasphyxiated	No fetal indication for intervention; repeat test weekly except in diabetic patient and postdate pregnancy (twice weekly)
8/10 normal fluid 8/8	Normal nonasphyxiated fetus	No fetal indication for intervention; repeat testing as per protocol
8/10 decreased fluid	Chronic fetal asphyxia suspected	Deliver
6	Possible fetal asphyxia	Deliver if AFV abnormal, if normal fluid at >36 wk with favorable cervix delivery; if ≤36 wk or L/S <2/1 or cervix unfavorable; repeat test in 24 hr; if repeat test ≤6 deliver; if repeat test >6 observe and repeat per protocol
4	Probable fetal asphyxia	Repeat testing same day; if BPS ≤6 deliver
0-2	Almost certain fetal asphyxia	Deliver

BPS = Biophysical profile scoring; AFV = amniotic fluid volume; L/S = lecithin/sphingomyelin ratio.

Table II. False-negative deaths and probability of fetal death after a normal last fetal biophysical profile score: Annual distribution

Study yr	*No. of patients (total)*	*No. of fetuses with last normal test*	*% of total patients*	*No. of false-negative deaths*	*Probability of false-negative fetal deaths/1000 patients*
1979-80	2586	2514	97.21	2	0.795
1981	2596	2526	97.3	2	0.791
1982	3199	3106	97.1	2	0.644
1983	3336	3249	97.4	2	0.555
1984	3751	3631	96.8	3	0.826
1985	4388	4265	97.2	3	0.703
Total	19856	19291	97.15	14	0.726

Method

Fetal biophysical profile scoring was the sole method used for fetal risk assessment in this study. The biophysical profile scoring is derived from the assessment of five variables (fetal movement, tone, breathing, qualitative amniotic fluid volume, and heart rate acceleration with fetal movement), which are coded as normal or abnormal according to fixed criteria and are then assigned an arbitrary score of 2 if normal and 0 when abnormal.[1] For the purpose of this ongoing study, a score of 8 or 10 was considered normal. A biophysical profile scoring of 8 was achieved in two ways: either four or five variables were normal, or more recently, if the first four variables monitored by dynamic ultrasound methods (movement, tone, breathing, and qualitative amniotic fluid volume) were normal. The latter modification has been studied prospectively and is found not to alter the predictive accuracy of the testing method.[5] When the test score was normal, it has been our consistent policy to advocate conservative therapy.[2] Exceptions to this policy are fetuses with a normal score and postdate pregnancies with a favorable cervix, growth-retarded fetuses with proved pulmonary maturity and a favorable cervix, insulin-dependent diabetic patients at 37 weeks or greater and proved pulmonary maturity, class A diabetic patients at term with a favorable cervix, patients with a previous abdominal delivery of a recurrent etiology and proved pulmonary maturity, and patients with unstable hypertension or other medical disorders that pose a serious threat to maternal health.

The study population was made up of consecutive referred pregnancies categorized as high risk by fixed referral criteria[2] and of at least 26 weeks' gestation. Fetal biophysical profile scores were obtained once weekly for all referred patients, except those with proved or suspected postmaturity (>42 completed weeks) and diabetes. The latter two groups were tested twice weekly. Test scores were used to guide management according to fixed criteria (Table I). The study period encompassed 75 months (1979 to 1985 inclusive).

A false-negative test result was defined as fetal death (stillbirth) that occurred in a structurally normal fetus within 7 days of a last normal test result (biophysical profile scoring = 8 or 10). Structurally normal severe Rh alloimmunized fetuses were excluded from this study population since death in such fetuses was caused by either the alloimmunization process or trauma at the time of therapy. The time of fetal death was determined from either maternal subjective notation of cessation of fetal movement or objective confirmation of the absence of fetal heart rate activity, whichever interval was least.

Table III. Death among structurally normal, nonisoimmunized fetuses within a week of a last normal fetal biophysical profile score

Case No.	*Initials*	*Referral risk factor*	*Fetal age at referral (wk)*	*No. of tests*	*Fetal age last test (wk)*
1	M. W.	Severe hypertension	27	3	28
2	F. H.	Peripheral vascular disease, myocardial infarction	27	4	29
3	G. T.	AP hemorrhage	28	2	30
4	L. S.	Sickle cell disease	30	2	31
5	V. Mc.	Alcoholic	31	2	32
6	K. F.	Decreased fetal movement	31	1	31
7	M. R.	Suspected IUGR	32	5	38
8	S. B.	Suspected IUGR	?32	2	32½
9	J. Mc.	Suspected IUGR	34	3	36
10	J. L.	Class A diabetes/suspect IUGR	36	1	36
11	D. O.	Hypertension	36	2	38
12	A. B.	Class A diabetes	37	2	38
13	M. M.	Hypertension	38	1	38
14	L. C.	Postdates	42½	1	42½

IUGR = Intrauterine growth retardation; AP = antepartum; IP = intrapartum; NST-R = reactive nonstress test.

Results

During the 75-month study period, 44,828 biophysical profile scoring results were recorded among 19,221 referred high-risk pregnancies. Considering multiple gestation, a total of 19,856 fetuses were studied. The primary indication for initial referral remained unchanged from previous reports[2] with the major indications being suspected intrauterine growth retardation (22.8%), hypertension (16.5%), postdate pregnancies (11.9%), and diabetes (9.4%). Both the number of patients and tests have increased annually, but the average number of tests per patient has remained relatively constant (Fig. 1). The mean fetal age at initial referral was 34 weeks (range, 26 to 44 weeks).

In total 44,828 fetal biophysical profile scores were recorded, 43,887 of which were normal (97.9%). A last normal test score was normal in 19,291 (97.15%) of the fetuses tested. Fourteen fetal deaths caused by factors other than major anomaly or alloimmunization were recorded. Therefore the probability of false-negative fetal death occurring within 1 week of a last normal fetal biophysical profile score was 0.726/1000 live births. This probability of false-negative fetal death varied on an annual basis, ranging from as low as 0.550/1000 to 0.826/1000 and did not change in accordance with the progressive increase in patients tested (Table II).

Clinical details of these 14 deaths, which are listed according to gestational age at initial referral, are given in Table III. Several points warrant additional comment. The mean interval between last test and fetal death was 4.43 days (range, 1 to 7 days), with two of 14 fetuses dying within 24 hours of a normal test result. Ten of the 14 fetuses had more than one normal score a week before the final test.

Four of the 14 deaths occurred during the intrapartum period. Two fetuses had continuous electronic fetal monitoring that documented evidence of fetal compromise. In one case (case 14) fetal distress was reported, but action was not taken for reasons that are unclear. In the other (case 13), chorioamnionitis was not treated in labor and the response to fetal distress was delayed. The other two intrapartum deaths followed overt cord prolapse.

Eight of 14 deaths (57.2%) occurred in the absence of recognized subsequent risk factors and are assumed to be the result of chronic asphyxia undetected at the time of testing; four of these deaths were associated with proved intrauterine growth retardation. The gestational age at which death was diagnosed ranged from 27 to 36 weeks. The mean birth weights were 1460 gm for the four fetuses with intrauterine growth retardation and 1622 gm for the other four. In the final two false-negative deaths, hypoxemic maternal deterioration (case 4) and acute placental abruptio (case 8) led directly to fetal demise, which was not predicted by biophysical profile scoring.

Two of the 14 mothers were diabetic, and four had diastolic blood pressure exceeding 90. There is no apparent relationship between risk factors prompting referral and the risk of false-negative fetal death in this group, of which the proportions for each category are similar to the entire referral population. Overall there is no apparent relationship between fetal age at last test and the risk of false-negative fetal death; the values ranged from 28 to 42½ weeks.

Comment

The end points used to judge test accuracy are variable. In this study we have used fetal death as the end

Last test result	*Interval test-death (days)*	*Birth weight (gm)*	*Time of death*	*Cause of death*
8/8	1	540	AP	Asphyxia unexplained, proved IUGR
8/8	3	830	AP	Asphyxia, unexplained
8/8	7	1100	AP	Asphyxia, unexplained
8/8	1	2200	AP	Maternal hypoxemia, sickle cell crisis
8/8	3	2160	IP	Acute asphyxia, cord prolapse at home
10 (NST-R)	4	1100	AP	Asphyxia, unexplained
8/8	7	2126	AP	Asphyxia, unexplained, associated IUGR
8/8	7	980	AP	Asphyxia, abruption, probable IUGR
8/8	7	1700	AP	Asphyxia, unexplained, associated IUGR
8/8	3	1470	AP	Asphyxia, unexplained, associated IUGR
10 (NST-R)	5	2480	AP	Asphyxia, unexplained
8/8	5	3920	IP	Acute asphyxia, cord prolapse
10 (NST-R)	5	3650	IP	Fetal distress, chorioamnionitis, fetal pneumonia
8/8	4	3400	IP	Fetal distress in labor, not treated

point, since unlike measures of morbidity this selected end point is not subject to interobserver variability or observer bias. We assume a relationship between the incidence of death and morbidity in a given study population, but this point was not specifically addressed in this study.

The calculated negative predictive accuracy for this biophysical profile scoring method was 99.224%; the reciprocal, that is, the probability of fetal death after a last normal test result, was 0.726/1000 patients. Therefore the high proportion of normal test results recorded with this method may be attributed to high negative predictive accuracy.

The ability to predict continued fetal survival for a finite interval has major clinical implications for both the mother and fetus. The art of obstetrics is based on the balancing of risks. For the high-risk pregnancy, this balance is usually between conservative management and the attendant risk of stillbirth and intervention with the attendant risks of prematurity complications and an increased probability of operative delivery. The high probability of continued fetal survival when the biophysical profile score is normal can be of major importance in reaching the appropriate management decision. Application of this concept to a specific high-risk population, postdate pregnancies, has been shown to reduce both fetal and maternal risks.[6]

The probability of fetal death after a last normal test, although low, would have been reduced even further under ideal conditions. The two cases of fetal death caused by cord prolapse would now be considered as ideally preventable in light of a recent report of an accurate antepartum method for the diagnosis of this condition.[7] It seems reasonable to assume that more aggressive intrapartum care would have prevented the deaths that occurred in labor. Therefore under the most ideal circumstances, the occurrence of false-negative fetal death could have been reduced to 0.518/1000 (10 deaths).

The perfect antepartum testing method would yield no false-negative deaths. Our cumulative experience with fetal biophysical profile scoring indicates that this test falls short of the ideal. An understanding of the basis for test failure is important to plan for either modification of the existing method, addition of ancillary variables, or both. The existing method is designed to evaluate fetal health at the time of assessment from which a statistical probability of continued fetal health may be constructed. The superimposition of either chronic deterioration in maternal or fetal condition or acute variables affecting condition will affect the accuracy of the testing method. The former constraint would be overcome by more frequent testing, but as shown in case 4, this modification would not prevent all false-negative deaths. Furthermore, whether the increased workload would be justified by a lower false-negative fetal death rate remains untested. Nonetheless, based on this experience we have now begun more frequent testing in the immature fetus with ultrasound-determined growth failure and have added proteinuric, hypertensive disorders to the indications for twice weekly biophysical profile scoring.

Recognition of premonitory signs of acute asphyxia would be a key step in improving test accuracy. The potential value of antenatal diagnosis of cord presentation is obvious. A method for the detection of impending abruption would be a great value. In this series one fetal death (case 8) and possibly at least one other death (case 1) might have been avoided by such information. The diagnosis of "abruption in evolution" by ultrasound methods is reported, but the field remains largely unexplored.[8]

We conclude from these data that a normal fetal biophysical profile score confers a high probability of subsequent fetal survival. In the clinical setting it would seem reasonable, based on these data, to continue conservative management of the immature fetus or the mother in whom delivery may not be accomplished with ease when the biophysical profile score is normal.

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Acute oligohydramnios in postdate pregnancy

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The evaluation of amniotic fluid volume plays a major role in antepartum fetal surveillance. Although the definition of diminished amniotic fluid volume varies, this sign is considered by itself an indication for intervention in the near-term fetus. The interval of testing is predicated on the concept that amniotic fluid volume diminishes slowly (unquantified) as a result of developing hypoxia. We present six postterm pregnancies in which amniotic fluid volume diminished abruptly over 24 hours. Although one fetus died, the three babies delivered by cesarean section and the two babies delivered after a 2-day labor did well, despite obvious postmaturity syndrome, meconium staining, and variable decelerations. Apgar scores in the survivors were above 8, and pH results failed to confirm hypoxia. These data suggest the need to better understand the mechanisms regulating amniotic fluid volume. (Am J Obstet Gynecol 1987;157:884-6.)

Key words: Acute oligohydramnios, postdate pregnancy, fetal surveillance

Oligohydramnios accompanies a broad range of reproductive disorders including anomalies of the fetus and functional disorders of the mother, fetus, and placenta.[1, 2] In nonanomalous fetuses, oligohydramnios may complicate postterm pregnancies and postmaturity syndrome in the newborn infant.

The recognition of these data have prompted some authors[1] to include assessment of amniotic fluid volume in the evaluation of fetal well-being as one of the components of the biophysical profile. Diminished amniotic fluid volume (however defined) generally mandates intervention (delivery) in the near-term fetus, irrespective of the results of other tests in the biophysical profile. Underlying this approach is the appealing but unsubstantiated allegation that amniotic fluid volume diminishes slowly (unquantified) in response to hypoxia or other stress and that the amniotic fluid volume will diminish before the fetus deteriorates. The assumption that amniotic fluid volume diminishes slowly has been challenged recently in the report by Weinbaum et al.,[2] who described a patient at 36 weeks of gestation in whom amniotic fluid volume diminished dramatically over 24 hours. We add to this observation by reporting on six women with postdate pregnancies in whom amniotic fluid volume also diminished dramatically over 24 hours.

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Material and methods

The maternal conditions, antepartum test results, delivery, and neonatal outcome data of these six patients are presented in Tables I and II. All patients (non-

Table I. Acute oligohydramnios in postdate pregnancy: Antepartum maternal and fetal test data

ID	*Age*	*G*	*P*	*Weeks' EGA*	*Test*	*Test no.*	*NST*	*AFV*
1	26	1	0-0-0-0	42 6/7	BPS	1	R	NOR
				43	BPS	2	R	ABS
2	22	2	1-0-0-1	41 4/7	BPS	1	R	NOR
				41 5/7	BPS	2	R	ABS
3	23	2	1-0-0-1	40 3/7	U/S	1		NOR
				41 1/7	U/S	2	R	NOR
				41 2/7	U/S	3	SB	ABS
4	23	1	0-0-0-0	41 1/7	BPS	1	R	NOR
				41 2/7	BPS	2	R	ABS
5	16	1	0-0-0-0	41 2/7	BPS	1	NR	NOR
				41 3/7	BPS	2	R	ABS
6	16	1	0-0-0-0	41 4/7	BPS	1	R	NOR
				42	BPS	2	NR	NOR
				42 1/7	BPS	3	R	ABS

ID = Identification number; G = gravidity; P = parity; EGA = estimated gestational age; NST = nonstress test; AFV = amniotic fluid volume; BPS = biophysical profile score; R = reactive NST; NOR = normal; ABS = diminished AFV; U/S = ultrasound; SB = stillborn; NR = nonreactive NST.

Table II. Acute oligohydramnios in postdate pregnancy: Delivery and neonatal data

ID	*Method of delivery*	*Sex*	*Birth weight (gm)*	*Apgar score*		*pH*	*HCT (%)*	*MEC*	*Outcome*
				1 min	*5 min*				
1	Cesarean	M	6152	9	9	7.27	57	Yes	Good
2	Cesarean	F	3728	8	9	7.31	55	Yes	Good
3	Vaginal	M	4125	0	0	—	—	Yes	Stillborn
4	Cesarean	F	3203	8	9	7.31	50	Yes	Good
5	Vaginal	M	4110	8	9	—	55	Yes	Good
6	Vaginal	F	3289	9	9	7.32	53	Yes	Good

ID = Identification number; HCT = hematocrit level; MEC = meconium staining.

smokers) had uneventful prenatal courses with normal glucose screening; each weighed well over 100 pounds and had gained weight normally during pregnancy. Each mother was tested initially because of potentially prolonged pregnancy (>287 days from the last menstrual period).

Results

We obtained biophysical profiles on 14 occasions in these six patients. Two patients received three tests; all others had two. In all cases the last two tests were obtained within 24 hours of each other. On the next to last test, four of the six fetuses demonstrated a reactive nonstress test (NST); two were deemed nonreactive. None revealed decelerations. Body movements, breathing movements, tone, and amniotic fluid volume (pocket >2 cm) were normal in each patient. All patients were examined on the following day. The two patients with nonreactive tests were examined as part of a routine follow-up, whereas those with reactive tests were seen because of complaints of decreased fetal movement. In all instances repeat examination revealed diminished and/or absent amniotic fluid volume (pocket <1 cm). In no instance was there evidence of extra amniotic collection of fluid or ruptured membranes. One of the babies with a previously reactive NST was dead; in the other five the NST was reactive, but variable decelerations were present.

On the basis of these findings, all patients had labor induced. Three (including the stillbirth) were delivered vaginally. Two babies were delivered uneventfully 2 days later, after prolonged induction of labor. Meconium-stained fluid and intermittent variable decelerations during labor were evident with these two babies, but they had average variability and reassuring scalp pH values. The three cesarean births were performed on the basis of variable decelerations during induction of labor.

The three male and three female infants ranged in birth weights from 3200 to over 6100 gm. Cord blood gas values were available in four of the five survivors and revealed normal pH values. The 1- and 5-minute Apgar scores for all of the survivors were ≥8. All five babies revealed stigmas of postmaturity syndrome, including meconium staining and evidence of wasting. The hematocrit level in three babies was elevated (>54%). However, all babies pursued normal courses and were discharged home with their mothers.

Comment

Oligohydramnios develops in about 0.5% to 4.0% of pregnancies, depending on the population, the interval between testing and delivery, and the criteria for diminished amniotic fluid volume. Case reports such as these do not permit the calculation of the frequency of acute oligohydramnios, the rate of disappearance of fluid, the volume of amniotic fluid mobilized, or the preexisting amniotic fluid volume. However, these reports may provide insight into the mechanisms of fetal and maternal homeostasis that result in redistribution of water from the fetal compartment. They also raise concerns about the scoring of biophysical parameters, the intervals between antepartum tests, and the value of including amniotic fluid volume as a critical fetal test parameter.

Despite the diminution in amniotic fluid volume, appearance of meconium, variable decelerations, and postmaturity syndrome in the five surviving babies, we found little obvious impact on the condition of the fetus. The cause of death could not be established in the case of the obviously postmature baby. On the basis of the normal antepartum tests 24 hours previously and the known association of oligohydramnios, cord compression (variable decelerations), and fetal distress and/or death, we infer that an abrupt cord accident followed the dramatic reduction in amniotic fluid volume and was the most probable cause of death. It seems unlikely that ongoing asphyxiation or even chronic hypoxia stimulates these dramatic changes in amniotic fluid volume. Indeed, all the surviving fetuses had variable decelerations during antepartum testing and early labor, but normal variability and pH values prevailed after the diminution in amniotic fluid volume, and two babies tolerated 36 to 48 hours of labor satisfactorily. Rayburn and others have previously commented on this syndrome of the nutritionally deprived but otherwise well-oxygenated fetus.

The postmaturity syndrome present in all of these babies draws attention to the fact that the loss of amniotic fluid volume indeed reflects fetal fluid loss. The dynamics of amniotic fluid exchange are complex, and the causes of transfer of water from the fetal to the maternal compartment have yet to be clearly defined. Fetal urine output and swallowing appear to be major determinants of amniotic fluid volume at term, and a number of factors associated with diminished urine production, such as hypoxia, may result in oligohydramnios. Nevertheless, there was little to suggest impaired urine output in any of the newborns reported here.

Studies of uterine and umbilical blood flow by Doppler examination were not available to us. If we are to understand the interrelationships of fetal condition, maternal and fetal blood flow, and amniotic fluid volume dynamics, such comprehensive testing will be necessary. Seemingly, logic dictates that uterine blood flow must be adequate to resorb so much amniotic fluid so quickly.

The scoring system proposed for the biophysical profile gives equal weight (2 points) to each variable (NST, breathing, tone, movement, volume). Advocates of the scoring system generally consider scores of 8 and 10 indicative of fetal well-being, requiring no clinical intervention. The cases reported here and elsewhere suggest that such scoring systems may be misleading. Further, the optimal interval for testing has not been defined. As a minimum, weekly testing seems inadequate in conditions in which the fetus is at risk of oligohydramnios (e.g., postdates or intrauterine growth retardation).

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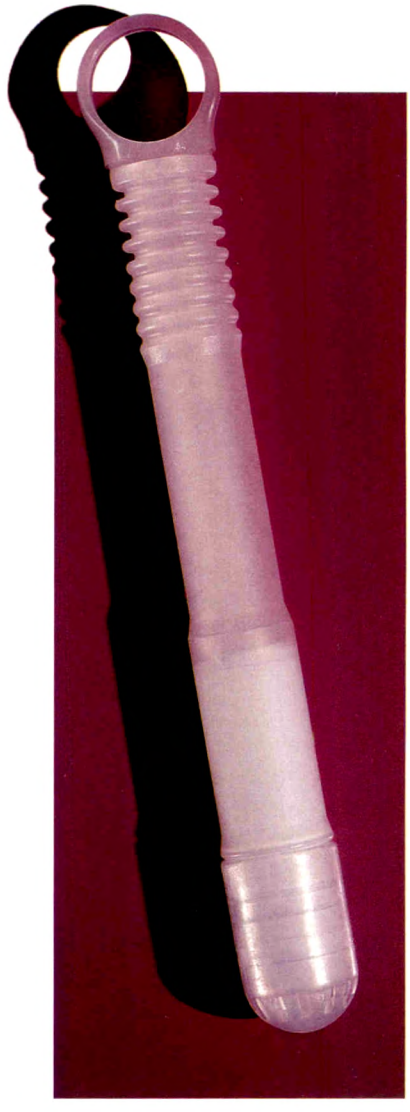
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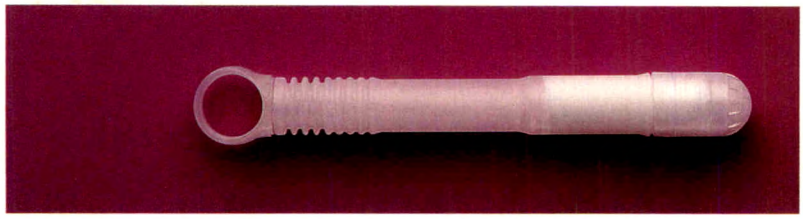
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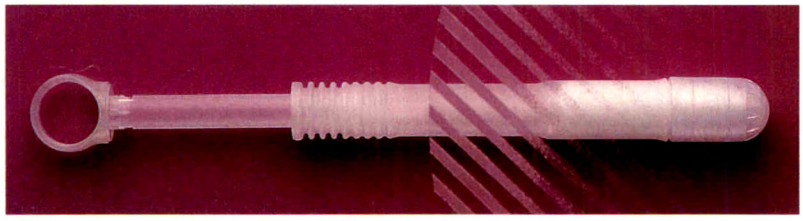
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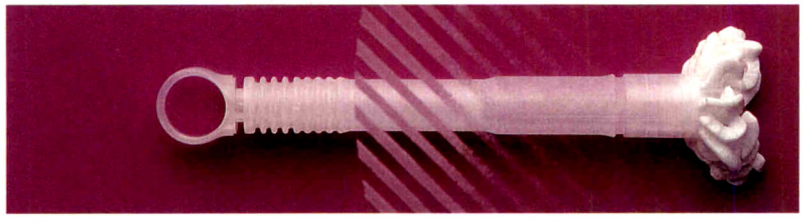
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1. Peel back protective foil to remove applicator. Do not attempt to remove special dispersion cap on tip of applicator.

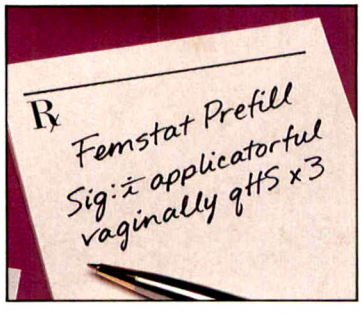


2. Insert applicator into vagina as far as possible without discomfort. Pull back on ring.



3. Push ring all the way forward to deliver medication. Withdraw applicator and discard.





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Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

February 1987

Abdominal pregnancy: Magnetic resonance identification with ultrasonographic follow-up of placental involution

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Omaha, Nebraska

Abdominal pregnancy is a rare event and preoperative diagnosis may be difficult. Recently, sonographic examination and magnetic resonance imaging have proved helpful in the preoperative diagnosis in a patient who presented with advanced abdominal pregnancy after tubal sterilization. As well, ultrasound was of benefit in the follow-up of placental involution after delivery. (AM J OBSTET GYNECOL 1987;157: 887-9.)

Key words: Abdominal pregnancy, placental involution, ultrasound, magnetic resonance

Abdominal pregnancy is a rare and dangerous obstetric phenomenon. The earliest description of an advanced abdominal pregnancy was recorded nearly one thousand years ago, and operative delivery with survival of mother and baby in such a case was accomplished by a swine gelder in Switzerland in the year 1500.

Preoperative diagnosis is often missed, usually because the rare condition has not been considered. Maternal history may be helpful, and previous pelvic inflammatory disease and ectopic pregnancy, together with abdominal discomfort in the absence of Braxton-Hicks contractions,[1] may be a guide. Sonography, which replaced abdominal film diagnosis, suggests the presence of an intra-abdominal pregnancy when an abnormal relation among fetus, placenta, amniotic fluid, and uterus is noted.[2] However, sonographic interpretation may occasionally be difficult because of distorted pelvic anatomy and/or overlying bowel gas. Recently, magnetic resonance imaging has been used in obstetric diagnosis, providing rather exact information regarding the relationship between the fetus, placenta, amniotic fluid, and uterus.[2]

We have had the opportunity to follow the late course of an abdominal pregnancy that was diagnosed by magnetic resonance imaging at 30 weeks' gestation by menstrual age. In addition, placental involution was characterized by ultrasound during an 8-month period after cesarean delivery of the fetus.

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Case report

A 24-year-old, gravida 4, para 3 woman was admitted to the hospital because of an enlarging, uncomfortable lower abdomen. The patient had felt some movement in the lower abdomen. She had had three previous uncomplicated pregnancies, the last delivery occurring 8 months before, after a normal labor and an uneventful puerperium. The patient had a menstrual period about 2 months thereafter, and dilatation, curettage, and laparoscopic tubal sterilization were performed. The patient consulted her physician again when she noted increasing abdominal size and abdominal discomfort.

A pelvic ultrasound evaluation revealed a fetus of approximately 30 weeks' gestation (menstrual age) by measurement of the biparietal diameter. A "mass" separate from the fetus was thought to be the uterus, a finding suggestive but not diagnostic of an extrauterine pregnancy. An x-ray film of the abdomen disclosed a single fetus in a transverse position with the fetal head on the maternal left. Magnetic resonance imaging confirmed the presence of an extrauterine pregnancy primarily on the left with a cephalad attachment of the placenta (Fig. 1). It could not be determined whether this attachment involved bowel, mesentery, omentum, uterus, or a combination. Because of the extrauterine location of the fetus, it was delivered by cesarean section. At operation the transverse position of a male fetus at the L-5 to S-1 level was confirmed. On delivery, Apgar scores were 5 at 1 minute and 8 at 5 minutes. The baby had multiple compressive deformities on the right parietal skull and the right mandible. Neck and chest asymmetry was noted with compressive deformities of the right side as compared with the left. Despite ventilatory support, the infant's pulmonary status deteriorated, and he died 10 days after delivery.

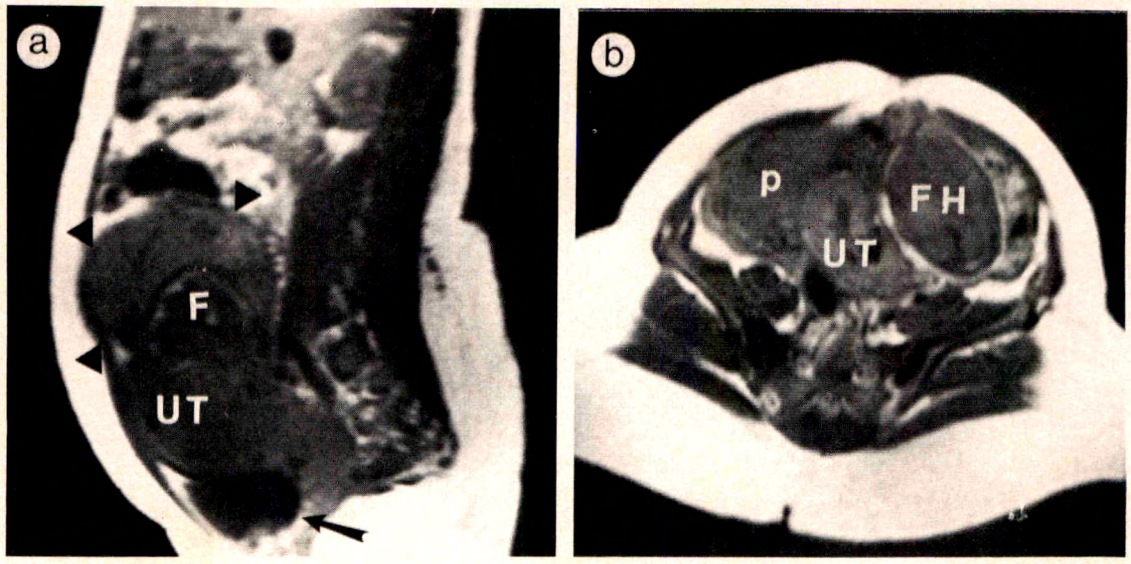


Fig. 1. Prepartum magnetic resonance images of maternal-fetal-placental structures. GE Signa System, 1.5 Tesla, T weight, General Electric. Partial saturation technique with TR of 800 and TE of 25. *a,* Sagittal view of the abdomen illustrating the position of the uterus *(UT)* with respect to the fetus *(F),* placenta *(arrowheads),* and bladder *(arrow). b,* An axial image of the abdomen confirms the relationship between the fetal head *(FH),* a portion of the placenta *(P),* and the uterus *(UT).*

The placenta was attached to the broad ligament, bowel, and mesentery; thus the umbilical cord was ligated proximal to the placenta after delivery of the baby and the placenta was left in situ. Pelvic ultrasonographic examination 2 weeks after delivery showed slight involution and decrease in the size of the placenta. The patient was followed up as an outpatient on a monthly basis with ultrasonography of the placenta to characterize the course of regression in placental size and vascularity. The most recent pelvic ultrasound evaluation 8 months post partum revealed an overall fourfold reduction in placental size since delivery.

Comment

Early abdominal pregnancy is typically self-limited by hemorrhage from trophoblastic invasion with complete abortion of the gestational sac, leaving a discrete crater that is sometimes difficult to identify.[3] Advanced abdominal pregnancies, as in our patient, survive the hemorrhage of trophoblastic invasion and implant secondarily during the first trimester on any adjacent structure.

The clinical picture of abdominal pregnancy is extremely variable, the most frequent signs and symptoms being abdominal pain (100%), vaginal bleeding early in pregnancy, persistent nausea and vomiting, general malaise, and painful fetal movements.[1, 2] A history of an acute, short-lasting episode of abdominal pain in early pregnancy, indicating a "tubal accident," is frequently recorded. Our patient had abdominal pain and experienced painful fetal movements.

Ninety-nine percent of all pregnancies are intrauterine. Of the 1% of pregnancies that are extrauterine, 98% are intratubal, 1% are ovarian, and 1% are primary or secondary peritoneal implants.[3] The primary abdominal type of extrauterine pregnancy is extremely rare. Classification of the primary abdominal pregnancy rests on the following criteria[1]: (1) Both tubes and ovaries are normal, with no evidence of recent or remote injury. (2) There is no evidence of uteroperitoneal fistulas. (3) The pregnancy is adherent to the peritoneal surface without evidence of secondary implantation after a primary nidation in the tube. In light of the tubal ligation performed 32 weeks before the cesarean delivery and apparently 2 weeks before implantation in our patient, the extrauterine pregnancy appears to be of the secondary abdominal type.

As alluded to above, the basis for abdominal pregnancy, whether primary or secondary, is only partially understood. At the time of ovulation, the mature ovum is covered with a sticky layer of granulosa cells that must be lysed before fertilization can occur. In some instances, the ovum may remain adherent to the follicle, become fertilized, and implant in the ovary as an ovarian pregnancy. The ovum may float freely in the peritoneal cavity, become fertilized, and gravitate into the cul-de-sac or any location in the peritoneal cavity.[3] Most secondary abdominal pregnancies result from tubal abortion or rupture, with extension and implantation of a viable placenta on the peritoneal surface. The occurrence of the secondary type (incidence of 1/2075 to

1/7931 deliveries) is influenced by previous pelvic infection, surgical adhesions, and tubal occlusion or improper use of antibiotics in pelvic inflammatory disease.[1] Such improper antibiotic use may produce a fibrotic tube, which will permit the passage of the sperm but which will not have a normal lining with peristalsis to permit the passage of the fertilized egg into the uterine cavity.[1,3] Abdominal pregnancy after tubal sterilization has been reported.

The clinical management of abdominal pregnancy is surgical regardless of gestational age or fetal condition, because of the otherwise high risk of placental separation and the incidence of congenital malformations. The maternal mortality rate for abdominal pregnancy is in the range of 4% to 18%.[1] The causes of death include massive hemorrhage caused by manipulating the placenta, sepsis, renal failure, and chronic blood loss. The fetal mortality rate is 90%, with an incidence of fetal deformity ranging from 1% to 100%.

Management of the placenta is variable and is determined by the placenta's location, blood supply, shape, and vascularity. Three different methods for dealing with the placenta are described[1,4]: (1) It can be removed; (2) it can be left in situ and allowed to resorb; (3) methotrexate therapy can be given to hasten resorption. Obviously, the placenta should be removed only when it is safe to do so. Complete removal has been reported in 71% of those patients reaching the third trimester of pregnancy. However, of the three choices available, leaving the placenta untreated or undisturbed now appears to be the most advisable, since the placenta commonly implants on adjacent vital organs and shares blood supply.[4] Under the latter circumstances, surgical removal of the placenta may compromise the blood supply to these organs. The use of methotrexate is limited by its potential for cytotoxic complications. Thus the best course of action, as in our patient, is to ligate the cord close to the placenta and leave it in situ.

Resorption of placental tissue is thought to be a slow process. Ultrasonographic follow-up in our patient showed a decrease in placental size to almost one fourth of its original volume during the first 7 months after delivery. No complication related to the placenta has occurred after 8 months of follow-up.

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Preterm birth at 23 to 26 weeks' gestation: Is active obstetric management justified?

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To determine if active perinatal management was justified in preterm deliveries at ≤26 weeks' gestation, the outcome (survival and short- and long-term morbidity) of 43 infants (group I) born between 23 and 26 weeks' gestation was compared with that of 17 infants (group II) born at 27 weeks' gestation. Of the 12 surviving infants in group I (survival 28%), 11 were ventilated (median = 50 days), eight had moderate to severe bronchopulmonary dysplasia, and four had mild retrolental fibroplasia. At follow-up, two infants had physical disabilities with moderate to severe functional impairment, one had a minor disability, and nine had normal neurodevelopment. In contrast, of the 13 surviving infants in group II (survival 76%), nine were ventilated (median = 8 days), six had moderate to severe bronchopulmonary dysplasia, and six had mild RLF. At follow-up, one infant had a physical disability with moderate to severe functional impairment, four had minor disabilities, and eight had normal development. Perinatal factors that positively influenced survival in the two groups combined included active perinatal management, antenatal steroids, female sex, and absence of clinical chorioamnionitis and asphyxia. Although group I infants had a significantly higher mortality rate ($p < 0.05$) and required a longer duration of ventilation ($p < 0.05$), no differences in the incidence of postnatal complications or long-term morbidity at 2 to 4 years of age were evident between the two groups. (Am J Obstet Gynecol 1987;157:890-7.)

Key words: Preterm delivery, active obstetric management, survival, neurodevelopmental outcome

Advances in obstetric and neonatal care and the improvement in survival rates and quality of outcome for very low birth weight infants in recent years have led to a reevaluation in the management of premature deliveries. More specifically, the management of deliveries between 23 and 26 weeks of gestation when the fetus has traditionally been regarded as nonviable, previable, or borderline viable has received increasing attention.[1-5] For perinatologists in tertiary care settings who must deal with these issues regularly and for primary care physicians who must decide whether to transfer such cases to tertiary care centers for further management, the crucial question is, what is the lowest gestational age that justifies active perinatal care? Below what gestational age should there be a hands-off approach by the perinatal team? In other words, how premature is too premature?

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At the Grace Maternity Hospital in Halifax, Nova Scotia, Canada (the major high-risk maternal referral center in Nova Scotia with 1000 high-risk deliveries out of 5400 annual deliveries), the approach has been to assume fetal viability and provide active perinatal management for pregnancies terminating at 27 weeks' gestation and above. With deliveries occurring at 26 weeks' gestation or less, there is no clear consensus. Most are treated passively, with regard primarily for maternal considerations.

Such an attitude is supported by previous reports on the outcome of infants born at 26 weeks' gestation or less, which indicate very high mortality rates and uniformly poor long-term neurodevelopment.[6, 7] Because more recent reports have demonstrated an improvement in survival rates and increasing numbers of intact survivors among these extremely preterm infants,[1, 2, 4] it has been suggested that the gestational age limit for active intervention on behalf of the fetus should be lowered even further—from the currently accepted 27 weeks to 24 weeks.[3]

We have reviewed our experience with this group of infants to determine if a change in approach is indicated. We reviewed the outcome of infants born between 23 and 26 weeks' gestation for: (1) survival, (2) short-term morbidity and (3) long-term neurodevel-

opmental status and compared it with that of infants born at 27 weeks' gestation during the same period.

Material and methods

The study population consisted of all liveborn infants delivered between 23 and 27 weeks of gestation within the Grace Maternity Hospital during the 3-year period from Jan. 1, 1980 to Dec. 31, 1982.

Gestational age was based on: (1) firm dates—a first-trimester history with known last normal menstrual period, regular periods, and no birth control pills used in the preceding 6 months and one or both of the following: (2) ultrasound examination in the first trimester with crown rump length agreeing with dates and (3) ultrasound examination between 18 and 20 weeks' gestation with biparietal diameter agreeing with dates, within 6 days.

Inclusion of patients into this study was made exclusively on the basis of assignment of prenatal gestational age. In cases in which there was a discrepancy between prenatal and postnatal assessments, prenatal gestational-age assessment prevailed. One infant with congenital anomalies incompatible with long-term survival has also been excluded from the study. Gestational age was assigned as the last completed week.

The obstetric managment of these patients varied. A key feature in the different approaches was the obstetrician's perception of fetal viability and the decision as to whether cesarean section would be considered evidence of fetal distress in labor or malpresentation (breech). When the fetus was thought to be nonviable by the obstetric staff, a hands-off approach was instituted. Many obstetricians who have made this decision would use no form of fetal monitoring; maternal well-being was the only consideration, with no intervention on behalf of the fetus (passive management). Those obstetricians who considered the fetus viable would attempt to deliver the fetus in the best possible condition, with electronic fetal monitoring used as a guide to timely operative vaginal delivery; others would perform a cesarean section for malpresentation or for fetal distress diagnosed by continuous electronic fetal heart rate monitoring, supplemented by fetal scalp blood pH (active management).

Other management decisions such as the use of tocolytics and/or steroids were largely based on the advancement of labor as indicated by dilatation of the cervix and whether amniotic membranes were ruptured, with gestational age not a crucial factor. Patients were classified as having received steroids if at least 24 hours had elapsed from the first dose to delivery. All obstetric management decisions were carried out under the direct supervision of attending obstetricians.

Neonatal staff attended all deliveries. Resuscitation was with bag and mask and oxygen or more commonly with endotracheal intubation. Each newborn infant was cared for in the special neonatal care nursery under direct supervision of a neonatologist.

Follow-up protocol

An appointment for assessment was offered to all surviving infants at the ages of 2, 3, or 4 years. All were seen at the Perinatal Follow-Up Clinic at the Grace Maternity Hospital, at which time an interim history was obtained and assessment of growth and physical and neurodevelopmental examinations were then carried out. Evidence of hydrocephalus, cerebral palsy, or minor changes in tone was noted. Psychometric assessment was performed by a clinical child psychologist who administered the Bayley Scales of Infant Development to the 2 year olds, and the McCarthy Scales of Children's Abilities to the 3 and 4 year olds, respectively. All scores and physical measurements were corrected for the infant's gestational age. Audiologic assessments were performed by the Nova Scotia Hearing and Speech Clinic, and if the child had not been seen recently by an ophthalmologist, an appointment was offered. None of the medical personnel participating in the follow-up protocol had participated in the perinatal management of these infants.

Definition of terms

Neonatal survival was defined as alive at discharge from the nursery.

Clinical asphyxia was defined as depression at birth that required positive pressure resuscitation for longer than 2 minutes, skin pallor, and abnormal neurologic signs (e.g., marked alteration of muscle tone or seizures).

Clinical chorioamnionitis was defined as malodorous vaginal discharge and/or fever (temperature >37.5° C), with lower abdominal tenderness before delivery. All cases of clinical chorioamnionitis were confirmed by histopathologic evidence of inflammation of the chorionic plate.

Bronchopulmonary dysplasia[8] was defined as follows: (1) respiratory distress beyond 2 weeks of age not otherwise explained and/or P_{CO_2} >60 mm Hg on two or more occasions beyond 1 week of age and (2) chest radiographic changes typical of bronchopulmonary dysplasia.[8]

Retrolental fibroplasia was graded as mild (grades 1 or 2) or moderate to severe (grades 3 and 4).

Physical disability was defined as cerebral palsy and visual or hearing deficits. Cerebral palsy was graded according to motor disabilities. Mild cerebral palsy was defined as a disability in ambulant children that interfered only slightly with normal daily functioning. Such children had hyperreflexia and hypertonicity, and if both lower limbs were affected, a positive Babinski re-

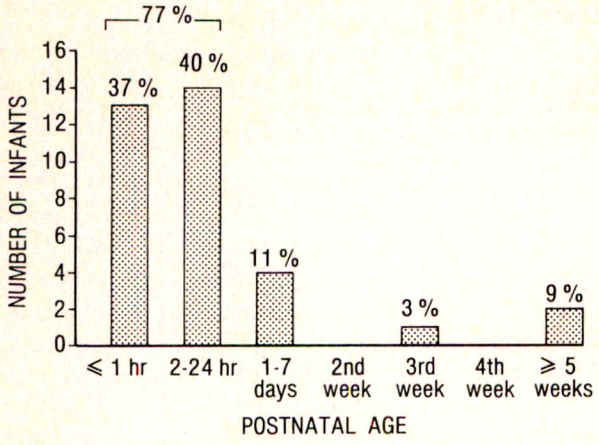


Fig. 1. Age at death.

flex. Moderate cerebral palsy included those children who attempted to walk at 2 years old. Severe cerebral palsy included those children who were likely to remain permanently nonambulant.[9]

Overall neurodevelopmental status was classified as normal or functionally handicap. An infant was considered normal if intellectual potential as measured by either the Bayley Mental Developmental Index or the McCarthy General Cognitive Index was in the normal range (i.e., above 84), and both gross motor and fine motor performances were qualitatively and quantitatively appropriate for the age. Functional handicap was classified according to the degree imposed by single or multiple physical disabilities (severe cerebral palsy, sensory impairment, and cognitive deficits). Severe functional handicap was considered to be present in children who had one or more of the following disabilities: moderate or severe cerebral palsy, a mental developmental index or a general cognitive index <69, and sensorineural deafness that required the use of hearing aids or bilateral blindness. A moderate functional handicap was diagnosed in children with a mental developmental index or a general cognitive index of 69 to 84 and one or more of the following: mild cerebral palsy, sensorineural deafness not requiring amplification, severe myopia, strabismus, or retrolental fibroplasia. Children were classified as having a mild functional handicap if they had either a mental developmental index or a general cognitive index score between 69 and 84 or mild cerebral palsy with a mental developmental index or a general cognitive index score >84.

Statistics

Continuous variables were analyzed by *t* test; discontinuous variables were analyzed by χ^2 test with the Yates correction or Fisher's exact test as needed. p Values <0.05 were considered statistically significant.

Results

Of the 14,776 infants delivered during the study period, 60 met the study criteria. All 60 mothers had a prenatal ultrasound examination in the first trimester and/or between 18 and 20 weeks' gestation. In 17 cases (28%), the crown rump length agreed with maternal dates, and in the remaining 43 cases (72%), the biparietal diameter agreed with the dates within 6 days. The birth weights of the 60 infants ranged from 450 to 1360 gm (mean ± SD = 746 ± 168 gm). Overall survival was 42% (25/60). The mean birth weight (±SD) of the 25 surviving infants was 810 ± 112 gm; for those who died it was 710 ± 187 gm ($p < 0.007$). The number and percentage of surviving infants and the birth weight distribution of the dead and surviving infants at each completed week of gestational age are shown in Table I. As expected, newborn survival at each week of gestation improved with increasing gestational age and was highest for infants at 27 weeks' gestation. The earliest survival was at 24 weeks' gestation (740 gm male). Of the 35 deaths, 13 deaths (37%) occurred within 1 hour of delivery (Fig. 1). An additional 14 infants (40%) died by 24 hours of age. The major causes of death were hyaline membrane disease (8/35, or 23%), extreme immaturity (6/35, or 17%), intrapartum asphyxia (6/35, or 17%), and infection (5/35, or 14%). Of the four infants who died after the first week of age, one infant (930 gm male born at 27 weeks) died at 15 days of age of massive pulmonary hemorrhage after a fulminant course of hyaline membrane disease. Two infants (a 780 gm female infant at 24 weeks and an 800 gm male at 27 weeks) died at 62 days and 168 days of age, respectively, of severe cystic bronchopulmonary dysplasia with pulmonary hypertension. The fourth infant (795 gm male at 25 weeks) died at 76 days of age from overwhelming sepsis, disseminated intravascular coagulopathy, and shock.

Survival

The survival rate in the infants who were 23 to 26 weeks' gestation (group I) was 28% compared with the 76% rate among infants who were 27 weeks' gestation (group II) (Table I). The birth weight and gestational age distribution of study infants in relation to mode of obstetric management is shown in Table II. Only 14 of the 43 infants (33%) in group I were born to mothers who had active obstetric management compared with 15 of 17 infants (88%) in group II. The earliest gestational age at which a cesarean section was performed was 25 weeks. Five patients (12%) received antenatal steroids at least 24 hours before delivery in group I compared with seven patients (41%) in group II. Of the perinatal factors evaluated for their influence on survival in group I infants, only female sex was positively correlated. However, when all study infants

Table I. Newborn survival and birth weight distribution at each completed week of gestation

Wk of gestation	*Total livebirths*	*Survivors*		*Birth weight (gm) (mean ± SD)*		
		No.	*%*	*Dead*	*Survived*	*Total*
23	7	0	0	560 ± 54	–	560 ± 54
24	10	1	10	460 ± 99	740*‡	650 ± 98
25	13	5	38	780 ± 274	704 ± 148*	751 ± 230
26	13	6	46	735 ± 185	813 ± 113*	771 ± 155
27	17	13	76	865 ± 75	854 ± 72*	857 ± 72
Total	60	25	42	710 ± 187	810 ± 112†	746 ± 168

*Not significantly different from those who died.
†Significantly different from those who died.
‡Lone survivor.

Table II. Birth weight and gestational age distribution in relation to mode of obstetric management

	Obstetric management				
	Active (n = 29)	*Passive (n = 31)*	*Active (n = 14)*	*Passive (n = 22)*	
Whole group					
Gestational age (mean ± SD)	26.2 ± 0.9	24 ± 1.3 ($p < 0.0001$)			
Birth weight (mean ± SD)	794 ± 136	701 ± 184 ($p < 0.03$)			
≤26 wk infants*					
Gestational age (mean ± SD)			25.3 ± 0.6	24.9 ± 0.8	NS
Birth weight (mean ± SD)			728 ± 160	731 ± 191	NS

*Twenty-four to 26 weeks' gestational age infants only.

(groups I and II combined) were considered, antenatal steroids, active obstetric management, absence of clinical chorioamnionitis or clinical asphyxia, and female sex were found to be positively correlated with neonatal survival (Table III).

Short-term morbidity

Table IV shows the frequency of postnatal complications in surviving infants born at 23 to 36 weeks and at 27 weeks' gestation. Major postnatal complications among surviving infants were bronchopulmonary dysplasia and retrolental fibroplasia.

Group I (infants 23 to 26 weeks' gestation). Of the 12 surviving infants born between 23 and 26 weeks' gestation, seven had idiopathic respiratory distress syndrome and required ventilatory support. Four additional infants in this group who had severe recurrent apnea also required ventilatory assistance. Not all infants were evaluated for intracranial hemorrhage by real-time ultrasonography, since this technique was not used routinely in our nursery until 1981. Of the nine infants in this group who had serial cranial ultrasound evaluation, five had no hemorrhage, two had hemorrhage into the germinal matrix, one had intraventricular hemorrhage with arrested ventriculomegaly, and one infant had bilateral intraparenchymal hemorrhage with hydrocephalus requiring ventriculoperitoneal shunt. Eight infants had moderate to severe bronchopulmonary dysplasia that required prolonged ventilation. Of the five infants in this group with patent ductus arteriosus, three required surgical ligation. All four infants with retrolental fibroplasia had only mild disease (grades 1 and 2).

Group II (infants 27 weeks' gestation). Of the 13 surviving infants born at 27 weeks' gestation, nine developed respiratory distress syndrome and required ventilatory support. Of the nine infants screened for intraventricular hemorrhage, only one infant had evidence of hemorrhage (germinal matrix hemorrhage only). Eight infants in this group had varying degrees of bronchopulmonary dysplasia, and all six infants with retrolental fibroplasia had only mild forms of the disease. Five of the nine infants with patent ductus arteriosus required surgical ligation of the duct.

There was no difference in the mean duration of hospital stay among the two groups of gestational-age infants.

Long-term follow-up (groups I and II)

All surviving infants were between 2 and 4 years of age at the time of developmental evaluation. Of the 12 survivors, in group I, six were two years old, five were 3 years old, and one was 4 years old, respectively, at the time of assessment. Of the 13 survivors in

Table III. Correlation of perinatal factors with neonatal survival

Characteristic	Percent survivor: Whole group n	Whole group %	p	≤26 wk only n	≤26 wk only %	p Value
Obstetric management						
Active	18/29	62	<0.005	7/14*	50	NS
Passive	7/31	23		5/22*	23	
Mode delivery (malpresentation)						
Vaginal	5/15	33	NS	3/13	23	NS
Cesarean section	2/4	50		1/2	50	
Antenatal steroids (>24 hr before delivery)						
Yes	8/12	67	<0.05	2/5	40	NS
No	17/48	35		10/38	26	
Clinical chorioamnionitis						
Yes	7/27	26	<0.025	4/22	18	NS
No	18/33	54		8/21	38	
Clinical asphyxia						
Yes	12/41	24	<0.005	8/33	24	NS
No	13/19	68		4/10	40	
Sex						
Male	9/36	33	<0.005	3/26	12	<0.005
Female	16/24	67		9/17	53	

*Twenty-four to 26 weeks' gestational age infants only.

Table IV. Comparison of postnatal morbidity in surviving infants born at ≤26 weeks' and 27 weeks' gestation

	≤26 wk (n = 12) (%)	27 wk (n = 13) (%)	p Value
Hyaline membrane disease	7 (58)	9 (69)	NS
Patent ductus arteriosus	5 (42)	10 (77)	NS
Clinical sepsis	10 (83)	8 (61)	NS
Ventilatory support (IMV only)	11 (92)	9 (69)	NS
Seizures	1 (8)	1 (8)	NS
Duration of IMV (median days)	50	8	<0.05
Age birth weight regained (days)*	14.6 ± 4.36	14.9 ± 6	NS
Length of hospital stay (days)*	135 ± 52	132 ± 89	NS
Retrolental fibroplasia grades 1 and 2	4 (33)	6 (46)	NS
Bronchopulmonary dysplasia			
Mild	0	3	NS
Moderate to severe	8	5	
Intra/periventricular hemorrhage	(n = 9)†	(n = 9)†	NS
Mild	2	1	
Moderate to severe	2	0	

IMV = Intermittent mandatory ventilation.
*Mean ± SD.
†Number screened by cranial ultrasound examination.

group II, seven were two years old, three were 3 years old, and three were 4 years old. Table V compares the long-term neurodevelopmental outcome in the two groups of infants. There was no statistically significant difference in the incidence of functional handicap or physical disabilities among the two groups of surviving infants. Two of 12 (17%) surviving infants in group I and one of 13 (7%) surviving infants in group II had physical disabilities.

The nature and severity of physical and functional handicap of affected infants are shown in Table VI.

Comment

For many decades, birth weight has been used as a convenient characteristic against which to measure survival prospects of premature infants. It was readily available, objective, and served to circumvent the difficulties of accurate determination of gestational age because of the uncertainties of the last normal menstrual period. Survival statistics based on birth weights largely served the interest of the neonatologist and had limited value to the obstetrician who must make the critical management decision before delivery. Although

Table V. Comparison of long-term outcome at ages 2 to 4 years between surviving infants born at ≤26 weeks' and 27 weeks' gestation

	≤26 wk (n = 12) (%)	*27 wk (n = 13) (%)*	*p Value*
Neurodevelopmental status			
Normal	9 (75)	8 (62)	NS
Minor functional handicap	1 (8)	4 (31)	NS
Moderate functional handicap	—	1 (7)	NS
Severe functional handicap	2 (17)	—	NS
Physical disability (CP, blindness, deafness)	2 (17)	1 (7)	NS

CP = Cerebral palsy.

Table VI. Survivors with functional ± physical disabilities

Gestation (wk)	*Birth weight (gm)*	*Sex*	*Cerebral palsy*	*Blindness*	*Sensorineural deafness*	*MDI/GCI*	*Functional handicap*
≤26 wk (n = 3)							
25	680	F	Spastic quadriplegia	Bilateral	—	Untestable	Severe
25	760	F	—	—	Severe	50	Severe
25	460	M	—	—	—	83	Mild
27 wk (n = 5)							
27	750	F	Mild spastic diplegia	—	—	80	Moderate
27	870	F	—	—	—	75	Mild
27	770	F	—	—	—	70	Mild
27	880	M	—	—	—	70	Mild
27	940	M	—	—	—	80	Mild

MDI = Mental Development Index. GCI = General Cognitive Index.

birth weight and gestational age have been shown to be major determinants of neonatal survival,[10] gestational age better reflects fetal maturation and is therefore more likely to be a better predictor of outcome. In a study of the survival of infants born at 24 to 28 weeks' gestation that used multivariate analysis, Herschel et al.[2] showed that gestational age made the most significant contribution to outcome; birth weight did not add to the discrimination. More recently, in a cohort of 1338 very premature and/or very low birth weight infants representing 94% of all liveborn infants at 32 weeks' gestation or less, Verloove-Vanhorick et al.[10] found that gestational age was a better predictor of neonatal survival than was birth weight. Therefore future studies on preterm infants are encouraged to place greater emphasis on gestational age, and not postnatal birth weight as the crucial independent variable affecting neonatal survival. Our neonatal survival rate at each completed week of gestation is comparable with that reported by others.[2, 6]

The survival of preterm infants partly depends on whether the obstetrician believes that the fetus is viable.[11, 12] Our aim was to determine if the nature of obstetric management (active or passive) at 23 to 26 weeks' gestation influenced neonatal survival. This was a difficult task, since the obstetrician's attitude does not readily lend itself to experimental investigation. Our definition of active obstetric management included all cases of assumed fetal viability in which the ultimate goal of management was to avoid fetal hypoxia and acidosis. This did not necessarily imply cesarean section. Other management maneuvers considered equally important included careful positioning of mother, generous use of oxygen in cases of suspected fetal distress, and avoidance of depressant drugs as much as possible during labor. In our institution during the review period, no delivery occurring earlier than 24 weeks was actively managed. Of the 14 deliveries between 24 and 26 weeks' gestation that had active obstetric management, seven infants (50%) survived. In contrast, only five of 22 infants (23%) born at 24 to 26 weeks' gestation whose mothers had not been actively managed in labor survived. Although this difference did not achieve statistical significance, there certainly was a trend for infants delivered to mothers who had been actively managed in labor to do better. However, when infants born at 27 weeks' gestation were included in the analysis, active intervention was clearly beneficial. Because many more infants were actively managed at 27 weeks' gestation (15/17, or 88%) than at ≤26 weeks (14/43, or 32%), the apparent difference observed in the analysis of the whole group may very well reflect the influence of gestational age and the mode of obstetric management.

Because very few mothers (5/43) received steroids at 26 weeks' gestation or less, the possible influence of this

management decision on neonatal survival at these gestations is difficult to determine.

The optimal mode of delivery for malpresentation in preterm infants remains controversial. Although some reports strongly favor the abdominal route,[1, 12] others are unable to show any impact of delivery method on neonatal survival.[13, 14] In a recent review of the impact of cesarean birth on preterm breech presentation, Bodmer et al.[15] concluded that although cesarean section was performed least often among extremely premature infants (25 to 28 weeks' gestation), these cases may prove most beneficial. During the period of our review, of 15 infants who were in breech presentations or with transverse lie at ≤26 weeks' gestation, only two (13%) were delivered by cesarean section. In contrast, two of four infants (50%) with malpresentation at 27 weeks' gestation were delivered by cesarean section. Because delivery by cesarean section for malpresentation was used more frequently when the infant was more mature, the improved survival advantage may only reflect gestational maturation. The advantage that was due to the method of delivery was unclear. Thus no conclusions can be drawn about abdominal delivery of infants at ≤26 weeks' gestation from this report because the numbers were too small.

It has recently been suggested that occult antepartum infection of the genital tract could be an important cause of preterm delivery.[16] Our data show a negative impact of clinical chorioamnionitis on neonatal survival for the whole group. The trend is similar for the infants born at ≤26 weeks' gestation but is not statistically significant (Table III).

Except for differences in the duration of ventilation, we were unable to demonstrate any other significant differences in postnatal morbidity (Table IV) between surviving infants born at ≤26 weeks' gestation and those born at 27 weeks. In this regard, our results contrast with those of Gilstrap et al.,[13] who reported significantly more serious postnatal morbidity among surviving infants born between 23 and 26 weeks' gestational age.

Earlier reports of long-term neurodevelopmental status of extremely premature infants showed uniformly poor outcome.[6, 7] Bennett-Britton et al.[6] reviewed the outcome of 158 infants weighing ≤800 gm at birth in a referral neonatal intensive care unit and found no intact survivors with gestational age ≤26 weeks or birth weight ≤700 gm. Our study population differs from that of Bennett-Britton et al.[6] in that it was inborn but is similar to that of Yu et al.,[4] who have recently reported more encouraging findings in gestational age groups identical to ours. Of 35 survivors born between 24 and 26 weeks' gestation, these investigators reported a 74% satisfactory outcome at 2 years of age. The present report has the advantage of a much longer period of follow-up and represents a 100% follow-up, with every survivor undergoing developmental assessment (Table V).

In summary, we have reviewed the outcome of infants born between 23 and 26 weeks' gestation (group I) in comparison with infants born at 27 weeks' gestation (group II). Group I infants had a significantly higher mortality rate, most notably at 23 and 24 weeks' gestation, and required a longer duration of ventilatory support. No other differences were evident between the two groups.

Analysis of perinatal factors showed that for groups I and II combined, active obstetric management, antenatal steroids, and female sex were associated with increased survival, whereas the presence of clinical chorioamnionitis and asphyxia resulted in increased death. When infants born at ≤26 weeks' gestation (group I) were analyzed separately, there was a trend for infants whose mothers had been actively managed in labor to have a better outcome, but this trend did not reach statistical significance.

It could be argued that intensive perinatal care should be widely extended to all deliveries occurring between 23 and 26 weeks' gestation, with the expectation that sooner or later perinatologists would become sufficiently adept to allow even the youngest infant to survive without major impairment. A prospective study designed with such an approach would have greater validity in addressing the gestational-age threshold question. However, the ethical, psychological, and economic implications of such an approach must also be addressed. Our data suggest a favorable long-term outcome for surviving infants born between 24 and 26 weeks' gestation but does not answer the question whether active perinatal management of these deliveries should become a standard practice. Further study is required to address this question.

The high neonatal loss among deliveries occurring between 23 and 26 weeks' gestations dictates that efforts aimed primarily at postponing deliveries beyond 26 weeks, or better still, eliminating premature delivery, should remain the mainstay of therapy. Until this is achievable, obstetric management decisions involving these deliveries will have to be made on an individual basis, keeping in mind that very preterm infants can have a favorable long-term outcome.

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Inhibitory effect of quinidine on plasma pseudocholinesterase activity in pregnant women

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The effect of quinidine at therapeutic and subtherapeutic concentrations on pseudocholinesterase activity in the plasma of 16 normal pregnant women was studied. The mean plasma pseudocholinesterase activity in the absence of quinidine (control) was 0.67 ± 0.11 U/ml. The mean pseudocholinesterase activity in the presence of quinidine at concentrations of 0.5, 1.0, 2.0, and 5.0 μg/ml was 0.48 ± 0.09, 0.38 ± 0.09, 0.29 ± 0.10, and 0.19 ± 0.09 U/ml, respectively. At therapeutic concentrations needed to treat cardiac arrhythmias (2 to 5 μg/ml), quinidine inhibited pseudocholinesterase activity by 60% to 70%. All the plasma samples had a normal dibucaine number (78 to 85). We recommend caution when succinylcholine and/or ester-type local anesthetics are used in pregnant women receiving quinidine. (AM J OBSTET GYNECOL 1987;157:897-9.)

Key words: Pregnancy, quinidine sulfate, plasma pseudocholinesterase

Pregnancy is associated with a significant decrease in plasma pseudocholinesterase activity.[1, 2] Pseudocholinesterase is responsible for the rapid metabolism of succinylcholine, a depolarizing muscle relaxant. Succinylcholine is still the most popular muscle relaxant used for rapid sequence intubation of the trachea in pregnant patients. Pseudocholinesterase is also responsible for hydrolysis of ester-type local anesthetics including procaine, tetracaine, cocaine, and chloroprocaine. Several pharmacologic agents interfere with the metabolism of these ester-type drugs by depressing pseudocholinesterase activity.[3-5] Quinidine, a potent antiarrhythmic agent, is known to prolong the muscle paralysis caused by succinylcholine.[6] The mechanism for prolongation of action of succinylcholine caused by quinidine is not well understood. Furthermore, the effect of quinidine at therapeutic concentrations (2 to 5 μg/ml) on pseudocholinesterase activity in pregnant women has not been reported. Recently we observed two cases of prolongation of succinylcholine duration of action in patients receiving quinidine. We also documented desensitization block after succinylcholine administration in a patient receiving quinidine sulfate. When measured after surgery pseudocholinesterase ac-

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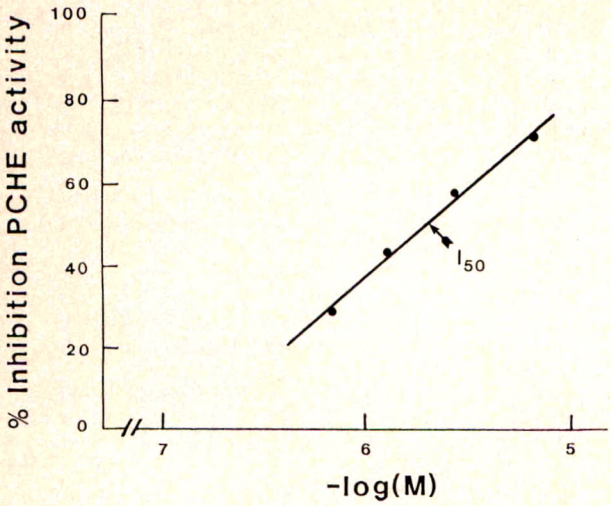


Fig. 1. Inhibitory effect of quinidine on plasma pseudocholinesterase activity. (I_{50} = 1.50 μg/ml; *PCHE* = pseudocholinesterase.)

tivity was significantly reduced in these patients. Therefore we studied the effect of quinidine on pseudocholinesterase activity in the plasma of 16 normal pregnant women.

Methods

With prior approval from our institutional review board for the protection of human subjects, we included 16 normal pregnant women (ages 18 to 35 years) in our study. Five milliliters of venous blood was withdrawn into a heparinized syringe from each women. Plasma was immediately separated and used for the determination of pseudocholinesterase activity and dibucaine numbers. A kinetic method described by Zapf and Coghlan[7] was used for the determination of pseudocholinesterase activity and dibucaine numbers. The effect of quinidine at concentrations of 0.5, 1.0, 2.0, and 5.0 μg/ml was also studied on pseudocholinesterase activity in each sample of plasma. Pseudocholinesterase activity was expressed as units per milliliter of plasma. One unit corresponds to the liberation of 1 μmol of 1-naphthol/min under the conditions of enzyme assay.

A randomized block analysis of variance within treatment levels and testing by the Dunnett test were used to compare the drug effects against the control. A difference was considered significant if $p < 0.05$.

Results

Table I contains the results of all the plasma samples. The mean pseudocholinesterase activity (control) of the pregnant women was 0.67 ± 0.11 U/ml. Pseudocholinesterase activity in the presence of quinidine at concentrations of 0.5, 1.0, 2.0, and 5.0 μg/ml was 0.48 ± 0.09, 0.38 ± 0.09, 0.29 ± 0.1, and 0.19 ± 0.09 U/ml, respectively. There was 29%, 43%, 57%, and 71% depression of pseudocholinesterase activity in the presence of quinidine at concentrations of 0.5, 1.0, 2.0, and 5.0 μg/ml, respectively. Dibucaine numbers of all the samples were in the normal range of 78 to 85.

Table I. Effect of quinidine on plasma pseudocholinesterase activity in pregnant women n = 16

Quinidine (μg/ml)	*Plasma pseudocholinesterase activity* (U/ml) (mean ± SD)*	*% inhibition of plasma pseudocholinesterase*
0 (control)	0.67 ± 0.10	—
0.5	0.48 ± 0.09	29
1.0	0.38 ± 0.09	43
2.0	0.29 ± 0.10	57
5.0	0.19 ± 0.09	71

*Significance of difference when compared with control; $p < 0.01$ (Dunnetts test).

Our results show a significant inhibitory effect of quinidine on pseudocholinesterase activity within treatment levels ($p < 0.001$). A Dunnett test clearly demonstrated a statistical difference in all treatment levels from the control ($p < 0.01$).

Fig. 1 shows a plot of percentage pseudocholinesterase inhibition against plasma quinidine concentrations. Linear correlation is evident as indicated by a correlation coefficient (R) of 0.99. Fig. 1 also shows that the plasma quinidine concentration required to inhibit 50% of the pseudocholinesterase activity was 1.50 μg/ml.

Comment

Pseudocholinesterase is a mucoprotein synthesized mainly in the liver. Both normal and preeclamptic pregnancies are associated with a significant decrease in pseudocholinesterase activity.[1, 2] The mechanisms for decreased pseudocholinesterase activity in pregnancy have not been elucidated.

Quinidine, a potent antiarrhythmic drug, is used to treat both atrial and ventricular arrhythmias. Quinidine is preferred to procainamide in the long-term management of arrhythmias because administration of procainamide is associated with drug-induced lupus erythematosus.

Our present data show that there is a significant decrease in pseudocholinesterase activity in normal pregnancy (0.67 U/ml) when compared with our normal mean value of normal nonpregnant women (0.9 U/ml). Our data also show that quinidine at concentrations needed to treat arrhythmias (2 to 5 μg/ml) depressed pseudocholinesterase activity an additional 60% to 70% from the control value of normal pregnancy.

We believe that it is important to be aware of this quinidine effect on pseudocholinesterase activity since systemic toxicity can occur after the epidural admin-

istration of ester-type local anesthetics. Systemic central nervous system toxicity after the administration of 2-chloroprocaine by means of an epidural has been documented in a young pregnant patient who apparently had less than 50% of normal level of pseudocholinesterase activity.[8] Moreover, because of the extremely low levels of pseudocholinesterase activity associated with therapeutic levels of quinidine, pregnant women should be monitored with a nerve stimulator for both the duration of action of succinylcholine and for desensitization block that may result after the administration of succinylcholine.

In conclusion, our data show that quinidine has a significant inhibitory effect on pseudocholinesterase activity in the plasma of pregnant women. Quinidine at therapeutic concentrations of 2 to 5 μg/ml depressed pseudocholinesterase activity by 60% to 70%. We recommend caution when succinylcholine and/or ester-type local anesthetics are administered to pregnant women who also receive quinidine.

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Umbilical artery flow velocity waveforms and the placental vascular bed

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Umbilical artery Doppler studies were performed close to delivery in 17 women (seven with healthy pregnancies and 10 complicated by intrauterine growth retardation), and results were analyzed by calculating the pulsatility index. In each case the placenta was examined histologically, and the small arteries and arterioles were counted in the tertiary stem villi. A highly significant negative linear relationship was found between pulsatility index and mean small arterial vessel count per field ($r = 0.71$, $p < 0.01$). This suggests that when the pulsatility index is raised (indicating increased resistance), one possible explanation may be obliteration of small placental arteries. (Am J Obstet Gynecol 1987;157:900-2.)

Key words: Umbilical artery, Doppler, flow velocity waveforms, placenta

Quantification of flow velocity waveforms of the umbilical artery obtained with Doppler ultrasound examination has become recognized as a potentially useful additional method of fetal assessment.[1] In adult vascular studies, end-diastolic velocity has been related to peripheral resistance.[2] Accordingly, a variety of distinct waveforms have been described in deep abdominal and pelvic arteries, with the characteristic of each dependent on the individual vascular bed.[3] In normal pregnancy there is a progressive increase in end-diastolic velocity[4] and hence a steady fall in peripheral resistance in the umbilical-placental circulation. Reduced, absent, or even reversed end-diastolic flow velocity may occur in pregnancies complicated by severe intrauterine growth retardation,[4] and such waveforms are associated with an increased risk of perinatal death[4] and low Apgar scores.[5]

Various hypotheses have been proposed to explain these changes, such as an increase in fetal whole-blood viscosity.[4] In a recent study Giles et al.[6] have demonstrated reduced mean small arterial vessel counts in placentas from pregnancies with abnormal flow velocity waveforms of the umbilical artery. They proposed that an increase in placental bed resistance caused by obliteration of these small vessels is the primary cause of the change observed in the flow velocity waveforms of the umbilical artery.

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Other investigators have not noted consistent morphologic changes in placentas from growth-retarded pregnancies.[7] This study was undertaken in an attempt to resolve some of this controversy. Changes in flow velocity waveforms were compared with the mean small arterial vessel count in the placenta from both normal pregnancies and those complicated by intrauterine growth retardation.

Patients and methods

Umbilical artery Doppler studies were performed in 17 pregnancies. Seven women were healthy with uncomplicated pregnancies and were delivered of babies who were appropriate for gestational age. All these women were studied within 3 days of birth. The other 10 women had pregnancies complicated by severe intrauterine growth retardation of varying etiology, and all were studied on the day of delivery. All birth weights were less than two standard deviations below the mean.[8] By studying the two diverse groups, it was hoped that a wide range of flow velocity waveforms of the umbilical artery would be obtained for analysis. A continuous-wave Doppler instrument (Bach-Simpson, London, Ontario, Canada) with a 4 MHz probe and a power output of 80 mW/cm^2 at the transducer surface was used in every case. The Doppler-shifted frequencies were processed by a spectral analyzer (Advanced Technology Laboratories, Bothwell, Wash.), and when the best auditory and visual signals were obtained, a hard copy printout was taken to include 10 waveforms, which were subsequently analyzed by calculating the pulsatility index as shown in Fig. 1.

The pulsatility index was selected as the optimal method of analysis, since a gradation of results can be obtained even when end-diastolic velocity is absent or

reversed whereas the systolic to diastolic ratio becomes infinity or negative, respectively. The pulsatility index results were not known by the pathologist examining the placental material.

Each placenta was studied by a modification of the method of Giles et al.[6] Immediately after delivery the umbilical cord was clamped, the placenta was weighed and measured, and then fixed in 10% neutral-buffered formalin for 3 to 4 days. The placenta was then cut longitudinally at 1.5 cm intervals, and five blocks of each placenta were selected at random for study. Samples taken from areas of infarction or within zones less than 2 cm from the cord insertion or the placental margin were excluded. The 2 cm zone beneath the cord insertion was excluded because of the finding by Giles et al.[6] that samples selected from this region were significantly different from other regions. Samples from the placental margin were excluded because of the high prevalence of intervillous fibrin deposition. The tissues were processed for histologic examination in the usual manner, cut at 5 μm thickness, and stained with hematoxylin-eosin and Gomori trichrome one-step stain.

The histologic analysis was performed with a camera lucida and an International Business Machine personal computer computer-assisted digitizing board. Five fields (× 100 magnification, 1.8 mm diameter) from the midzone of each section were studied (25 fields/placenta). Areas of placental infarction, intervillous fibrin deposition, and primary stem and anchoring villi were excluded. We counted the number of arteries, arterioles, and tertiary stem villi in each field. All results were expressed as the mean value per field for each placenta.

Regression analysis was performed, and the correlation coefficient, p value, and r square values were determined.

The mean gestational age of the intrauterine growth retardation group was 31 weeks ± 3 days while that of the control group was 37 weeks ± 5 days. The mean birth weight in the intrauterine growth retardation group was 1053 ± 459 gm and 3054 ± 1245 gm in the control group.

Results

The mean small arterial vessel count was determined in all cases, and a highly statistically significant linear association was found between increasing pulsatility index and reduced mean arterial count ($r = 0.71$ $p < 0.01$). The results are demonstrated in Fig. 2. The r square value was 0.50, which indicates that 50% of the variation seen in the pulsatility index may be explained by mean small arterial vessel count. As might be expected, the highest pulsatility index values and lowest mean small arterial vessel counts in the placenta

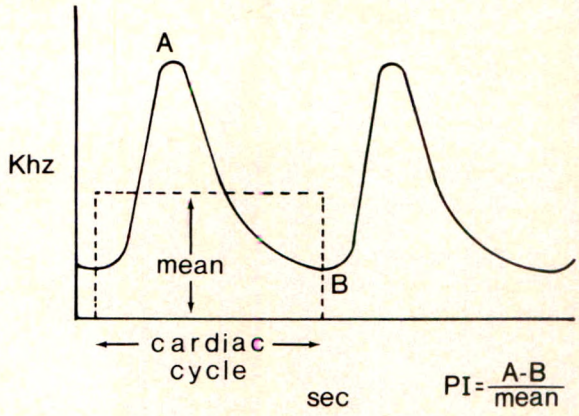


Fig. 1. Schematic flow velocity waveform indicating the method for calculating the pulsatility index *(PI)*.

were obtained when the fetus was growth retarded, whereas in normal pregnancies the pulsatility index values were low and mean small arterial vessel counts were high.

A modal count of the small arterial vessels was also determined in all cases so that a comparison could be made between our results and those of Giles et al.[6] This comparison could not be completed because eight of the 19 cases studied had more than one modal value.

A strong negative correlation was found in this study between the pulsatility index and mean small arterial vessel count, thus when the pulsatility index is elevated (indicating increased peripheral resistance), mean small arterial vessel count is also reduced. This suggests that the abnormal flow velocity waveforms of the umbilical artery may be at least partly explained by the reduced numbers of these vessels, which are the prime regulators of peripheral resistance.[9]

There was no difference in tertiary stem villous count between patients with normal and abnormal pulsatility index values and hence the reduced mean small arterial vessel count seen in some cases cannot be explained on the basis of a reduced number of tertiary stem villi.

The results of this study confirm the findings of Giles et al.[6] who initially reported reduced numbers of small placental arterial vessels in women with abnormal umbilical flow velocity waveforms. From our study it was not possible to say whether the reduced number of vessels was because of failure of development or obliteration. Giles et al.[6] observed collections of nuclear material in some abnormal placentas, which they felt may have represented obliteration of a small artery. An obliterative process would be supported by the fact that in some pregnancies flow velocity waveforms of the umbilical artery may be normal initially and become progressively abnormal with advancing gestation.

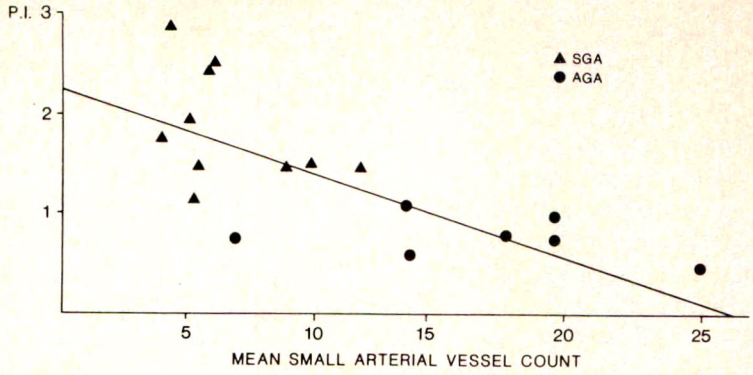


Fig. 2. Correlation between pulsatility index and mean small arterial vessel count ($r = 0.71, p < 0.01$). (*SGA* = Small for gestational age; *AGA* = appropriate for gestational age.)

The mechanism leading to this suspected obliteration is not known. However, the most likely hypothesis suggested by Rankin and McLaughlin[10] is that reduced uteroplacental perfusion results in uteroplacental ischemia, which leads to placental vasoconstriction. If this process was prolonged or severe, obliteration of the vessels could occur.

In summary, a highly significant linear correlation was found between flow velocity waveforms of the umbilical artery quantified by the pulsatility index and the mean placental small arterial vessel count. This suggests that when the pulsatility index is raised (indicating increased resistance), the major cause is obliteration of small placental arteries.

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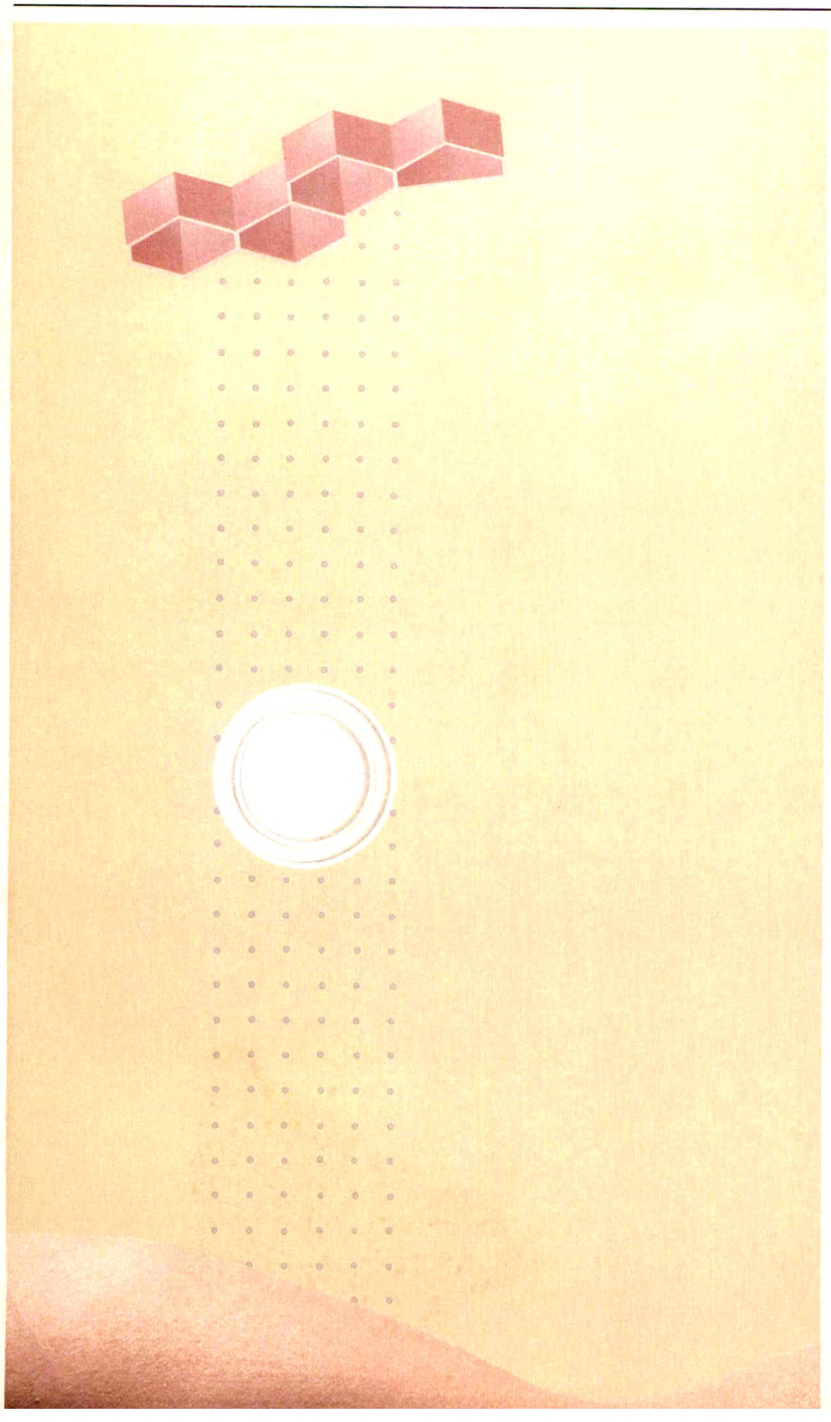
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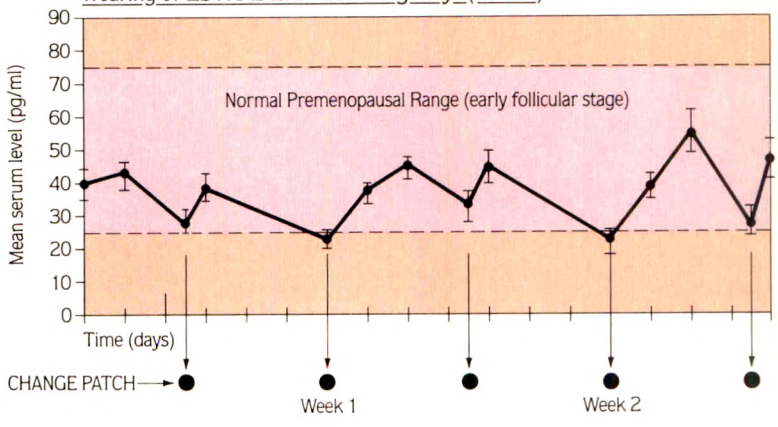
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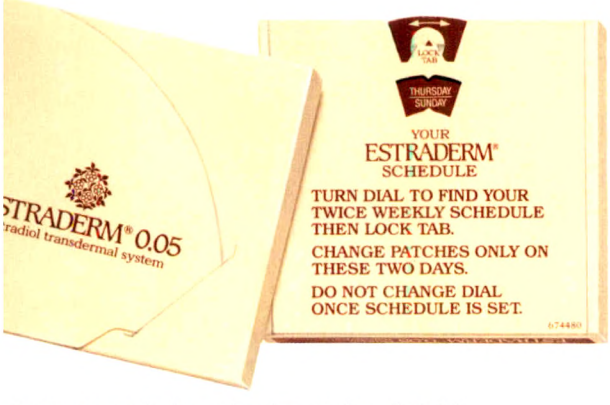
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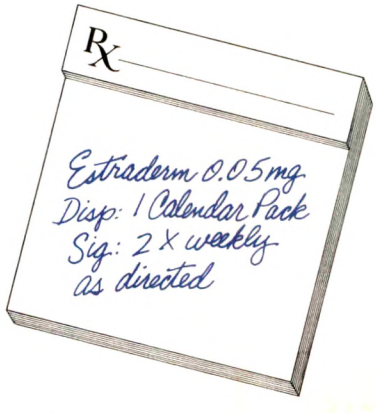
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ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than 1 year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer-reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5-13.9 times greater than in nonusers. The risk appears to depend both on duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that women who had been exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than four per 1000 exposures. Furthermore, a high percentage of such exposed women (30-90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data on the use of other estrogens are not available, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7-fold increased risk of limb-reduction defects in infants who had been exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estraderm is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and of the advisability of continuation of the pregnancy.

INDICATIONS AND USAGE

Estraderm is indicated for the treatment of the following: moderate-to-severe vasomotor symptoms associated with menopause; female hypogonadism; female castration; primary ovarian failure; and atrophic conditions caused by deficient endogenous estrogen production, such as atrophic vaginitis and kraurosis vulvae.

Studies of the addition of a progestin for seven or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of endometrium suggest that 12 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks that may be associated with the inclusion of progestin in estrogen replacement regimens. The potential risks include adverse effects on carbohydrate and lipid metabolism. The choice of progestin and dosage may be important in minimizing these adverse effects.

CONTRAINDICATIONS

Estrogens should not be used in women or men with any of the following conditions:

1. known or suspected cancer of the breast;
2. known or suspected estrogen-dependent neoplasia;
3. known or suspected pregnancy (see Boxed Warning);
4. undiagnosed abnormal genital bleeding;
5. active thrombophlebitis or thromboembolic disorders;
6. history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. Induction of Malignant Neoplasms. Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time, there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of breast cancer, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. Gallbladder Disease. A recent study has reported a two- to threefold increase in the risk of surgically confirmed gallbladder disease in postmenopausal women receiving oral estrogens, similar to the twofold increase previously noted in users of oral contraceptives.

3. Effects Similar to Those Caused by Estrogen-Progestogen Oral Contraceptives. There are several serious adverse effects of oral contraceptives and other high-dose oral estrogen treatments, most of which have not, up to now, been documented as consequences of postmenopausal estrogen replacement therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women.

a. Thromboembolic Disease. It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least four weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. Hepatic Adenoma. Benign hepatic adenomas have been associated with the use of oral contraceptives. Although benign and rare, these tumors may rupture and cause death from intra-abdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations, but they should be considered if abdominal pain and tenderness, abdominal mass, or hypovolemic shock occurs in patients receiving estrogen. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The causal relationship of this malignancy to these drugs is not known.

c. Elevated Blood Pressure. Women using oral contraceptives sometimes experience increased blood pressure which, in most cases, returns to normal upon discontinuing the drug. There is now a report that this may occur with use of oral estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used. Ethinyl estradiol and conjugated estrogens have been shown to increase renin substrate. In contrast to these oral estrogens, transdermally administered estradiol does not affect renin substrate.

d. Glucose Tolerance. A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. Hypercalcemia. Administration of high doses of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If hypercalcemia occurs, use of the drug should be stopped and appropriate measures should be taken to reduce the serum calcium level.

PRECAUTIONS

General

1. A complete medical and family history should be taken before initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, as well as a cervical Papanicolaou test. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.
2. Because estrogens may cause some degree of fluid retention, careful observation is required when conditions that might be influenced by this factor are present (e.g., asthma, epilepsy, migraine, and cardiac or renal dysfunction).
3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as uterine bleeding, mastodynia, etc.
4. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Estrogens should be used with caution in patients who have or have had endometriosis.
5. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.
6. Preexisting uterine leiomyomata may increase in size during prolonged estrogen use. If this occurs, estrogen therapy should be discontinued while the cause is investigated.
7. In patients with a history of jaundice during pregnancy, there is an increased risk that jaundice will recur with the use of estrogen-containing oral contraceptives. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.
8. Estrogens may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.
9. Because the prolonged use of estrogens influences the metabolism of calcium and phosphorus, estrogens should be used with caution in patients with metabolic bone diseases associated with hypercalcemia and in patients with renal insufficiency.

Drug/Laboratory Test Interactions

The results of certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following changes have been observed with large doses of oral estrogen:

1. increased sulfobromophthalein retention;
2. increased prothrombin time; increased factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability;
3. increased thyroxine-binding globulin (TBG), leading to increased circulating total thyroid hormone (T_4) as measured by column or radioimmunoassay; free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered; TBG was not affected in clinical trials of Estraderm;
4. reduced response to the metyrapone test;
5. reduced serum folate concentration;
6. increased serum triglyceride and phospholipid concentration, and decreased pregnanediol excretion.

The pathologist should be informed that the patient is receiving estrogen therapy when relevant specimens are submitted.

Carcinogenesis, Mutagenesis, Impairment of Fertility

See **WARNINGS** and Boxed Warning.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

Pregnancy Category X

See **CONTRAINDICATIONS** and Boxed Warning.

Estrogens should not be used during pregnancy.

Nursing Mothers

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

See **WARNINGS** and Boxed Warning regarding potential adverse effects on the fetus, induction of malignant neoplasms, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.

The most commonly reported adverse reaction to Estraderm in clinical trials was redness and irritation at the application site. This occurred in about 17% of the women treated and caused approximately 2% to discontinue therapy. Reports of rash have been rare.

The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

Genitourinary System: Breakthrough bleeding, spotting, change in menstrual flow; increase in size of uterine fibromyomata; change in cervical erosion and amount of cervical secretion.

Endocrine: Breast tenderness, breast enlargement.

Gastrointestinal: Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice have been observed with oral estrogen therapy.

Eyes: Steepening of corneal curvature; intolerance to contact lenses.

Central Nervous System: Headache, migraine, dizziness.

Miscellaneous: Change in weight, edema, change in libido.

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Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

No other OC regimen phases *both* hormones

▼

Provides the *lowest* monthly dose of both estrogen and progestogen

▼

Little or no effect on lipid metabolism[1-5]*

▼

Low overall incidence of breakthrough bleeding[6]

*Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These data represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to all data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

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TRI·LEVLEN™ 28
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Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

BRIEF SUMMARY

Tri-Levlen™—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

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430-34

Adenosquamous carcinoma of the fallopian tube

A clinicopathologic case report with verification of the diagnosis by immunohistochemical and ultrastructural studies

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Chapel Hill, North Carolina

A middle-aged woman presented with a pelvic mass. Pathologic examination of the resected specimen revealed a primary adenosquamous carcinoma of the left fallopian tube. Special studies supported the concept of the neoplastic cells differentiating along two major pathways, squamous cell carcinoma and mucin-producing adenocarcinoma. (Am J Obstet Gynecol 1987;157:903-5.)

Key words: Fallopian tube neoplasia, adenosquamous carcinoma, keratin immunohistochemistry, electron microscopy

Fallopian tube cancer is the rarest of gynecologic malignancies, with an incidence of less than 1%. We report a case of primary fallopian tube carcinoma with a heterogeneous cell population showing pathways of differentiation toward both squamous cell carcinoma and adenocarcinoma.

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Case report

A 42-year-old white woman, gravida 2, para 1-0-1-1, presented with a 3-week history of left lower-quadrant abdominal pain, radiating into the left leg and a concomitant 7-pound weight loss. Pelvic examination revealed a 9 cm firm, exquisitely tender mass at the vaginal apex extending posteriorly toward the cul-de-sac and to the left of midline. Her past medical history was significant in that she had a total vaginal hysterectomy

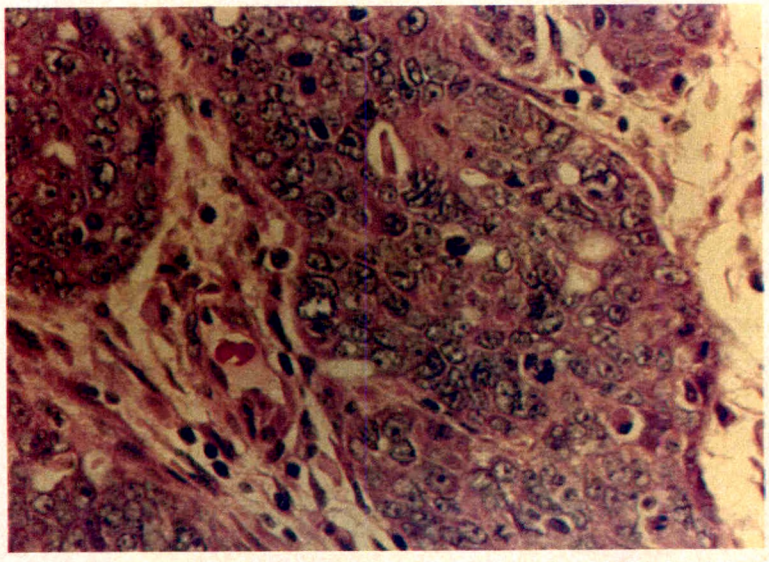


Fig. 1. Representative field of predominantly nonkeratinizing squamous cell carcinoma. Attempts at lumen formation are minimal. (Hematoxylin and eosin. Original magnification ×160.)

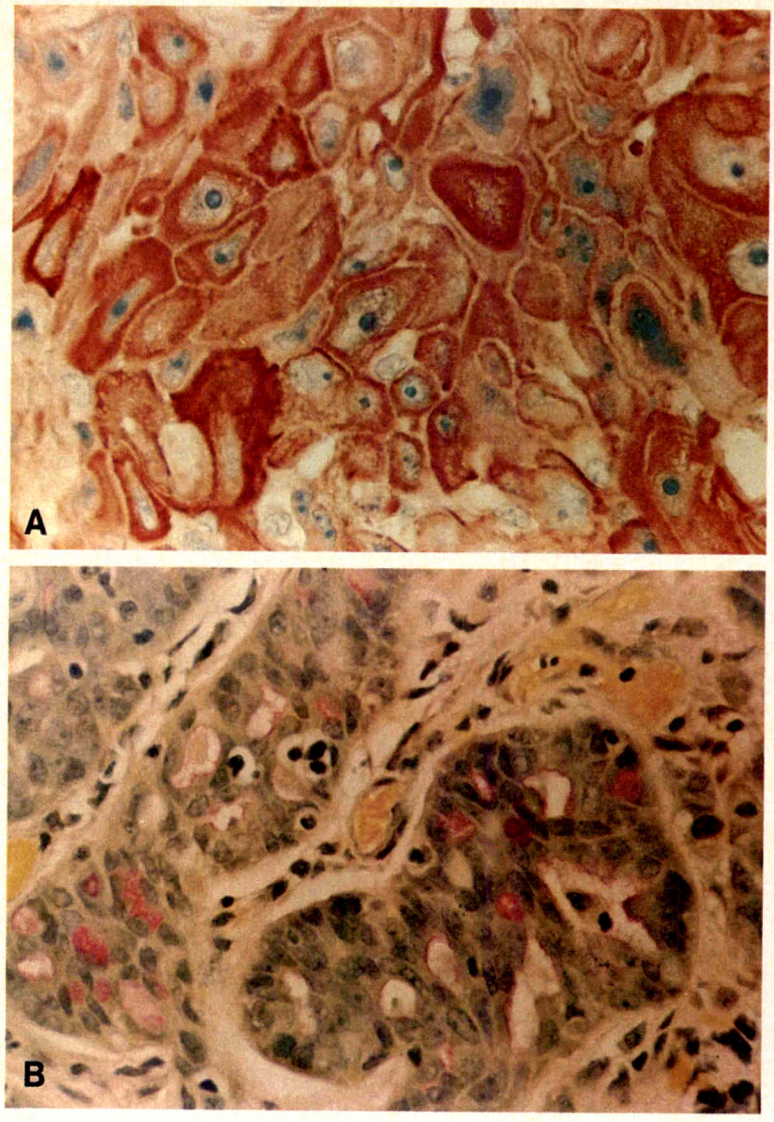


Fig. 2. *A*, Intense cytoplasmic immunoreactivity of keratinizing squamous neoplastic cells with polyclonal antibodies against keratin(s). (3,3′-Diaminobenzidine with hematoxylin counterstain. Original magnification ×250.) *B*, Intracytoplasmic and extracellular mucins are seen within islands of neoplastic cells similar to those demonstrated in Fig. 1. (Mucicarmine–metanil yellow. Original magnification ×160.)

performed 20 years earlier. Pathologic examination of the uterus revealed chronic cervicitis and squamous metaplasia of the endocervical epithelium.

The presence of a total leukocyte count of 23,300 cells/mm^3 in association with the findings described above led to a diagnosis of a pelvic abscess. She was treated with intravenous gentamicin and clindamycin. A pelvic computed tomographic scan showed an 8 by 9 cm "complex" left pelvic mass, without ascites or adenopathy. Other laboratory and radiologic studies were noncontributory. Her symptoms rapidly resolved with treatment and she was discharged on a regimen of a 1-week course of oral antibiotics. She was then readmitted for exploratory laparotomy, bilateral salpingo-oophorectomy, partial omentectomy, and pelvic and periaortic lymph node biopsies. A mass arising from the left adnexa and impinging on the sigmoid colon and cul-de-sac peritoneum was resected. She had an unremarkable postoperative course. Cytopathologic examination of peritoneal washings showed no evidence of malignant cells.

The left adnexal mass was an irregular, bosselated, spheroid structure (10 by 7 by 7 cm), appearing grossly to arise from the enlarged fallopian tube and expanding into the peritubal soft tissue. A normal-appearing left ovary was adherent to the surface of the mass.

Sections of the mass showed extensive necrosis with solid areas of moderately differentiated keratinizing

and nonkeratinizing neoplastic squamous cells (Figs. 1 and 2, *A*). The neoplastic cells were immunoreactive with antibodies directed against human stratum corneum (predominantly 56 and 64 kD) keratins (Immunon Corp., Troy, Michigan). Juxtaposed sections of the fallopian tube showed a lumen filled with a neoplasm with an "endometrioid" histologic pattern without evidence of squamous differentiation. These cells also demonstrated "positivity" with the antikeratins. Intracellular and extracellular mucins were identified histochemically (mucicarmine) and keratin was also found immunohistochemically at the site where the neoplasm extended out through the fallopian tube wall (Fig. 2). The sections of the ovary showed only superficial invasion by squamous cell carcinoma.

Transmission electron microscopy of the tumor showed both glandular and squamous differentiation. Intraluminal gland formation was prominently seen (Fig. 3). Intermediate filaments consistent with tonofilament (keratin) bundles along with desmosomes and microvilli were easily identified. The cytoplasm of the tumor cells contained prominent rough endoplasmic reticulum, glycogen, and amorphous material.

Comment

Fallopian tube malignancies, because of their rarity, are usually not diagnosed before definitive surgical treatment. In retrospect, most patients sought medical attention because of abdominal pain, a pelvic mass, or vaginal bleeding. Histologically, these tumors are almost always adenocarcinomas and are usually unilateral. However, like endometrial neoplasms, tubal adenoacanthoma has been reported. Mallinak et al.[1] reported a case of a pure tubal squamous cell carcinoma and Imm[2] described a tubal adenosquamous carcinoma on the basis of routine light microscopic observations.

Keratins are a family of at least 19 polypeptides that are organized as intermediate filaments in all epithelia. Many tumors, including squamous cell carcinomas and adenocarcinomas of the genitourinary tract, have been found to be immunoreactive under appropriate conditions of fixation and antibody selection. So-called endometrioid adenocarcinomas, as a group, are usually devoid of intracellular mucin. However, those arising in the fallopian tube have not been studied in large enough numbers for us to know if the positivity seen in our case is an exception to the usual rule. Our report confirms the existence of this entity and supports the concept that müllerian-derived epithelium can give rise to lesions with multipotential cytologic features in the fallopian tube.

We thank Dr. F. B. Askin for reviewing the histopathologic material.

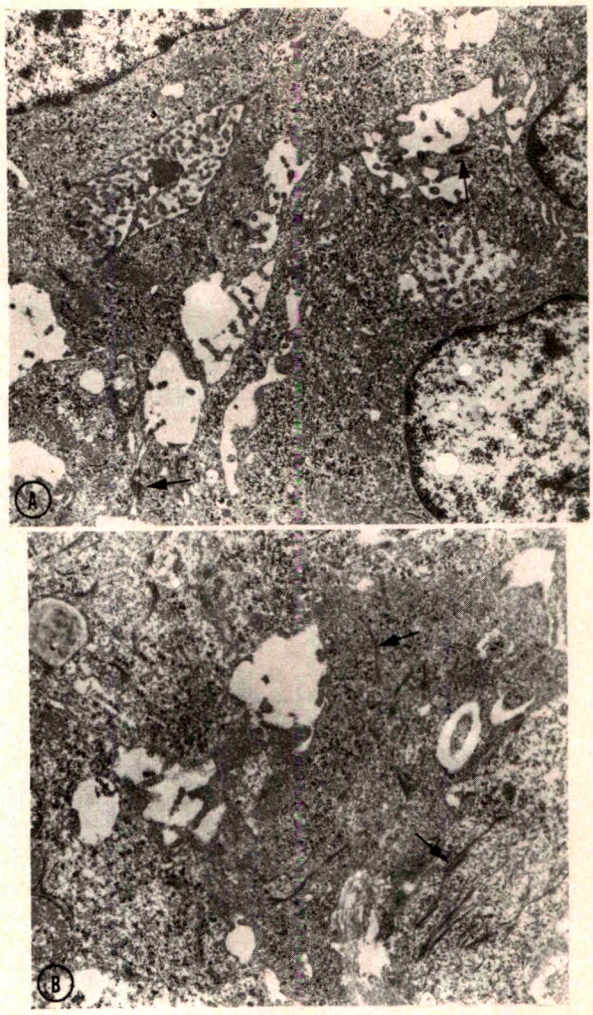


Fig. 3. Transmission electron micrograph demonstrating glandular lumina lined by microvilli. *A*, Well-formed desmosomes are appreciated at cell contact points *(arrows)*. (×6800.) *B*, A high-power view of the tonofilaments that were also present ultrastructurally in many cells throughout the tumor (×13,000.)

Addendum

After receiving two courses of cisplatin chemotherapy (50 mg/m^2), the patient was lost to follow-up for 5 months. She then presented with a histologically verified pelvic recurrence that was resected. No extrapelvic spread was identified at operation, and she is currently undergoing further cisplatin chemotherapy.

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Increased tubular enzyme excretion in preeclampsia

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The pathophysiology of preeclampsia includes ischemia and microinfarctions of the kidney, which could induce renal tubular cells to release enzymes into urine. We therefore measured the concentrations of two markers of renal tubular damage, *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase, in urine specimens from women with mild or severe preeclampsia and compared the results with those from healthy pregnant and nonpregnant women. The median urinary concentrations of *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase in women without preeclampsia increased progressively through the first, second, and third trimesters and reached maximum values of 1.12 and 0.77 U/mmol creatinine, respectively. Median concentrations of the two enzymes were significantly higher in women with mild preeclampsia (*N*-acetyl-β-D-glucosaminidase = 1.40, alanine aminopeptidase = 1.12 U/mmol creatinine) or severe preeclampsia (*N*-acetyl-β-D-glucosaminidase = 2.90, alanine aminopeptidase = 1.26 U/mmol creatinine). This increased enzyme excretion indicates subclinical preeclamptic renal tubular damage. (AM J OBSTET GYNECOL 1987;157:906-8.)

Key words: Normal pregnancy, preeclampsia, *N*-acetyl-β-D-glucosaminidase, alanine aminopeptidase, renal tubules, urine

Preeclampsia-eclampsia is associated with high rates of maternal and fetal mortality and morbidity.[1] Hypertension and vasospastic ischemia leading to multiple microinfarctions of the brain, liver, and kidney are characteristic features of the disease.[2] Damage to these organs accounts for other common manifestations: edema, proteinuria, and cerebral dysfunction.

In the kidney, the classic lesion is glomerular endotheliosis. Renal tubular damage may also be a feature of preeclampsia. We have evaluated subclinical renal tubular damage in patients receiving anticancer drugs by monitoring the excretion of the renal enzymes *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase.[3-6] Since urinary levels of *N*-acetyl-β-D-glucosaminidase are increased in patients with renal artery stenosis[7] and essential hypertension,[8, 9] we reasoned that preeclampsia might similarly induce the release of enzymes from tubular cells into urine. By measuring urinary concentrations of *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase, we sought to quantify the extent of tubular damage in women with mild or severe preeclampsia.

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Patients and methods

Urine specimens were obtained from healthy nonpregnant women and from healthy women in their first, second, and third trimesters of pregnancy. Specimens were also obtained from women who met the criteria for mild or severe preeclampsia.[1] Mild preeclampsia was defined as a rise in blood pressure that is 30 mm Hg systolic or 15 mm Hg diastolic above baseline readings on two separate occasions at least 6 hours apart. Severe preeclampsia was defined as the presence of blood pressure of 160/110 mm Hg on two separate occasions, 6 hours apart, accompanied by proteinuria with or without generalized edema.

Specimens were stored at 4° C and centrifuged to remove amorphous salts before analysis. The urinary concentrations of alanine aminopeptidase and *N*-acetyl-β-D-glucosaminidase, which were determined by automated spectrophotometric methods,[3, 10] were expressed relative to the urinary creatinine concentration to adjust for variations in urine output.[11]

The Kruskal-Wallis nonparametric analysis of variance test with adjustments for multiple comparisons as proposed by Nemenyi,[12] was used to compare median concentrations among groups of patients.

Results

The six study groups consisted of 10 nonpregnant women (mean age, 21.6 years ± 4.6 SD), 34 normotensive pregnant women in the first trimester (mean age, 19.3 years ± 3.9 SD), 26 in the second trimester (mean age, 18.3 years ± 4.2 SD), and 40 in the third trimester (mean age, 19.5 years ± 4.0 SD). Thirty women with mild preeclampsia (mean age, 18.7

years ± 4.1 SD) and 46 with severe preeclampsia (mean age, 19.1 ± 4.3 SD) were also in the third trimester. Gestation times did not vary significantly between women with preeclampsia and normotensive women in the third trimester, and all pregnant women were primigravidas. Two patients in the group with severe preeclampsia had thrombocytopenia, hemolysis, and elevated liver enzymes.

Fig. 1 shows urinary concentrations of *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase for women with preeclampsia compared with pregnant and nonpregnant control subjects. Enzymuria in healthy pregnant women was clearly increased over findings in nonpregnant women. The *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase levels showed progressive increases with gestation time; the median values for both markers in healthy third-trimester women were significantly higher than those for women in the first trimester ($p < 0.05$). The median *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase levels for women with mild or severe preeclampsia were significantly higher than the median value for healthy pregnant women in the third trimester ($p < 0.05$).

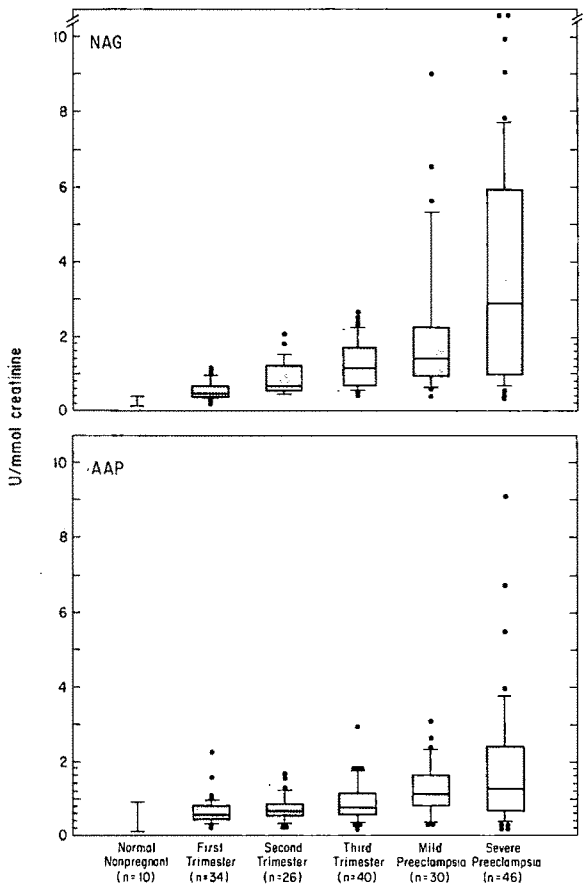


Fig. 1. Urinary *N*-acetyl-β-D-glucosaminidase *(NAG)* and alanine aminopeptidase *(AAP)* concentrations in women with preeclampsia and control subjects. For each category, the *shaded box* includes the median value *(bar)*, with ranges to the twenty-fifth and seventy-fifth percentiles; the *vertical bars* extend to tenth and ninetieth percentiles, with all other values shown individually. For nonpregnant women, the *vertical bar* indicates the range of values.

Comment

The increased excretion of *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase, as well as other enzymes,[11, 13] in pregnant women stems from normal changes in renal function that enhance the rate of enzyme turnover. However, *N*-acetyl-β-D-glucosaminidase levels in women with preeclampsia were higher than those associated with normal pregnancy or those reported in essential hypertension.[8, 9] The increased enzyme excretion in women with preeclampsia may reflect renal ischemia and relative hypertension of pregnancy. The striking enzyme excretion in some women with severe preeclampsia suggests a tubular lesion consonant with arteriolar constriction and microinfarction.

Many of the low values for *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase in women with preeclampsia in this study might be ascribed to fluctuations (intermittent vasospasms) in the intensity of the disease. Moreover, the diagnosis of preeclampsia is difficult to establish with certainty on clinical grounds alone.[12] However, patients with preeclampsia whom we studied were young primigravidas without any history of chronic hypertension or renal disease. Moreover, 68 of the 76 patients had normal blood pressures before 20 weeks' gestation. Hence, it can be argued that most of these patients had pure preeclampsia.

The overlap of values for *N*-acetyl-β-D-glucosaminidase and alanine aminopeptidase in women with preeclampsia with those for healthy women in the third trimester precludes any diagnostic use for single specimens. The clinical value of monitoring enzyme concentrations is limited to the detection of nonspecific tubular damage because excretion is increased in patients with other renal diseases and by administration of many drugs. Nevertheless, longitudinal monitoring of selected patients at risk of developing the disease may be of value for investigations of subclinical renal tubular ischemia. Ischemic tubular damage may precede the development of glomerular endotheliosis and clinically overt preeclampsia.

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Short course of antibiotics for post–cesarean section endometritis

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Although post–cesarean section endometritis is a common postoperative complication, little has been reported on the safety of short-course therapy, particularly in indigent populations. Our preliminary data on 25 indigent patients with endometritis treated only until 24 to 36 hours after defervescence suggest that courses of antibiotics need not routinely be continued for 7 to 10 days. (AM J OBSTET GYNECOL 1987;157:908-9.)

Key words: Cesarean section, endometritis, antibiotics

Endometritis is one of the most common complications of cesarean section. Although modern antimicrobial therapy has helped to reduce the sequelae of infection, the increasing operative rate makes endometritis a continuing, common clinical problem. Much literature exists dealing with the relative merits of various antibiotic regimens, but little has been published on the appropriate duration of therapy.

The common practice of continuing antibiotics arbitrarily for 7 to 10 days (switching from parenteral to oral, if there is an oral equivalent, when the patient has been afebrile for 48 to 72 hours) has recently been questioned.[1] However, scant data have been reported concerning the safety and efficacy of shorter therapeutic courses of antibiotics. The data that have been published have not provided microbiologic information and have reported only on a generally "healthy" population, leaving open the question of the utility of this approach in indigent populations in whom infection rates are generally higher and host resistance potentially reduced.[2] In an attempt to assess the safety of a short course of antibiotics in an indigent population with defined pathogens, we evaluated 25 service patients diagnosed as having post–cesarean section endometritis (uterine tenderness and standard fever).

Material and methods

Patients were black and Hispanic and of lower socioeconomic status (either medicaid or no insurance). Endometrial cultures were obtained in all patients with triple-lumen catheters before initiation of single-agent

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antibiotic therapy (imipenem, 500 mg every 8 hours). Patients were treated with intramuscular medication until they had been afebrile for 24 to 36 hours. No follow-up oral medications were given. All patients underwent final evaluations 4 to 6 weeks after discharge.

Results

Pretherapy cultures yielded 53 isolates, 38 of which were high virulence. Twenty-one of the 25 patients responded to imipenen alone. One patient had phlebitis, one had a bladder flap hematoma, and two required additional antibiotics before defervescence. In all cases therapy was discontinued 24 to 36 weeks after the patient had been afebrile. The mean duration of therapy, including primary treatment failures, was 4.4 days. However, what is most important is that 16 of 25 patients required 4 or fewer days of antibiotics whereas only the four patients for whom treatment failed required therapy beyond 5 days. Thus 65% of patients received less antibiotics then they would have if therapy had been given routinely for 5 or more days. Further, 13 patients completed the course of treatment on the fourth or fifth postoperative day. If the course of antibiotics had been extended to complete a predetermined course, the hospital stay would have had to be prolonged (there is no oral equivalent for imipenem).

All patients were well on discharge and at the time of follow-up. There were no readmissions.

In an article summarizing post–cesarean section endometritis, Duff[1] wrote, "Parenteral therapy should be continued for 24 to 48 hours after the patient becomes afebrile and asymptomatic. At this point antibiotics may be discontinued." Despite the common sense of this advice, at the time it was written no documentation of the safety of a shortened course of antibiotics existed. Indeed, studies comparing the efficacy of various therapeutic regimens frequently advised continuing antimicrobials for an arbitrary period independent of the time of defervescence. The need to heed Duff's advice is heightened at a time of increased scrutiny of medical care costs that would be clearly raised by courses of antibiotics continued for 7 to 10 days rather than the 4.4 days used in this study. This would be especially problematic if the antibiotic used had no oral equivalent in which case hospital stay also might be prolonged. In 1987 Soper et al.[2] documented the safe application of a short course of antibiotics in a dependent military population. They cautioned that the applicability of their approach to an indigent population needed to be tested. Further, no microbiologic data were provided to document infection with high-virulence organisms. Our preliminary report confirms their findings and suggests that their conclusions may also be applicable in an indigent population infected with high-virulence organisms. Clearly larger numbers of patients need to be examined, but our initial data support the call of Duff and Soper et al. for shorter courses of therapeutic antibiotics.

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First-trimester serum levels of the β-subunit of human chorionic gonadotropin in a tubal molar pregnancy

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A woman is described in whom the levels of the β-subunit of human chorionic gonadotropin were rising in a pattern consistent with a tubal pregnancy. A tubal molar pregnancy was found. Thus an early ectopic molar pregnancy is not distinguishable from a nontrophoblastic tubal pregnancy on the basis of human chorionic gonadotropin levels. (Am J Obstet Gynecol 1987;157:910.)

Key words: Tubal molar pregnancy, human chorionic gonadotropin

Frequently the serum human chorionic gonadotropin (hCG) β-subunit levels in ectopic pregnancy do not show an appropriate doubling time.[1] The hCG β-subunit levels in patients with gestational trophoblastic disease are generally higher than anticipated for conceptual dating.[2] A case is presented of a tubal molar pregnancy, in which serial hCG titers were closely monitored shortly after conception. The levels were not distinguishable from hCG patterns seen in ectopic gestations of nontrophoblastic proliferation.

Case report

A 38-year-old nulligravid woman with a history of long-term infertility and oligomenorrhea had ovulation induction with clomiphene citrate, 50 mg, on days 5 to 9. Two mature follicles of 18.3 mm (right ovary) and 19.3 mm (left ovary) formed, and the functional maturity of those follicles was evidenced by a serum estradiol level of 655 pg/ml with a serum progesterone level of 0.3 ng/ml. Release of the ova from the follicles was also documented by sonography. The patient presented 5 weeks after the documented release of the ova but the hCG β-subunit level was only 83 mIU/ml. However, the hCG level proceeded to rise such that 9 days later it was 883 mIU/ml. It then rose to 903 mIU/ml 4 days later and reached a peak of 3347 mIU/ml 19 days later. At this time a pelvic ultrasound evaluation failed to reveal a gestational sac and instead demonstrated a 16 by 12 by 13 mm echo-free area in the right adnexa with no free fluid seen. A diagnostic laparoscopy revealed a leaking right tubal pregnancy involving the right proximal and mid ampullary segment. The left adnexa appeared totally within normal limits and at this point a right partial salpingectomy was performed. The pathologic diagnosis on the resected tubal segment revealed hydatidiform mole with moderate trophoblastic proliferation arising in the ectopic pregnancy within the tube. Pathologic consultation was requested from a member of the trophoblastic disease registry who also reached a similar diagnosis, hydatidiform mole arising in an ectopic (oviductal) gestation and chronic salpingitis. The patient's postoperative course was uneventful and she permitted determination of only two follow-up hCG levels: 10.6 mIU/ml 13 days after operation and <5 mIU/ml 21 days after operation.

Comment

The failure to show appropriate doubling time of the hCG β-subunit level, from an initial value of 83 mIU/ml to only 903 mIU/ml 14 days later, is consistent with a typical pattern seen with tubal pregnancy. Thus, had the day of conception not been known, the interpretation of these hCG levels would not make one suspicious of trophoblastic disease. However, the marked rise in the hCG β-subunit level despite a previous low level of 83 mIU/ml 5 weeks after conception (which had suggested spontaneous abortion) created great suspicion of trophoblastic proliferation.

After 55 days from conception an appropriate interval doubling of the hCG β-subunit was seen for the first time and one can only speculate that had there not been surgical intervention that a more rapid inappropriate elevation of the hCG levels might have been noticed.

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Pregnancy in Henoch-Schönlein purpura

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A 22-year-old white woman with Henoch-Schönlein purpura was treated with frequent plasmapheresis for increased severity of the disease activity during pregnancy. Hypertension, azotemia, and placental dysfunction were not observed. A healthy infant was delivered by cesarean section, and evidence of vasculitis was not detected in the placenta. (Am J Obstet Gynecol 1987;157:911-2.)

Key words: Pregnancy, Henoch-Schönlein purpura, plasmapheresis

Henoch-Schönlein purpura is a clinical syndrome of generalized vasculitis manifested by purpuric skin rash, hematuria, proteinuria, abdominal pain, gastrointestinal bleeding, and arthralgias. The treatment of Henoch-Schönlein purpura, including the use of corticosteroids, has been unrewarding. Plasmapheresis has been tried in a few patients for deteriorating renal failure with varying success.

Very little is known about pregnancy in Henoch-Schönlein purpura. Thus far, only two women with Henoch-Schönlein purpura during pregnancy have been described.[1,2] We describe herein the successful use of frequent plasmapheresis for the treatment of recurrent and severe attacks of Henoch-Schönlein purpura during pregnancy.

Case presentation

A 22-year-old white nulliparous woman was admitted to the hospital in January 1982 with nausea and abdominal pain. There was no rash, fever, or gastrointestinal bleeding. Results of laboratory studies were normal except for proteinuria and hematuria. Ultrasonography of the abdomen revealed gallstones. Symptomatic relief was achieved after cholecystectomy 4 months later.

In February 1983, she was hospitalized with a 2-week history of purpura, fever, abdominal pain, and hematuria. She denied joint pain, melena, or headache. Crops of nontender palpable purpura, 1 to 3 mm in diameter, were present on the abdomen and legs associated with right upper quadrant tenderness. Urinalysis revealed a trace of protein and 75 to 100 erythrocytes but no leukocytes. Routine chemistries, blood cell count, and coagulation test results were normal. The skin biopsy specimen revealed leukocytoclastic vasculitis with immunoglobulin G and immunoglobulin M deposits. Circulating immune complexes were elevated (43.5 μg antihuman globulin Eq/ml by Clq binding assay); C3, C4, and CH-100 were low. A clinical diagnosis of Henoch-Schönlein purpura was made. A 2-week trial of steroids resulted in no symptomatic improvement. Subsequently, her symptoms gradually subsided but recurred in June 1983. Plasmapheresis with removal of 2 L plasma and replacement with 1 L normal saline solution and 1 L 5% albumin was performed on 2 consecutive days. The abdominal pain responded promptly to the first plasmapheresis and no new purpura appeared after the second.

The patient was admitted to the hospital six more times in the next 22 months and was treated with plasmapheresis each time with rapid symptomatic improvement. She remained symptom free between relapses but microscopic hematuria persisted.

The patient became pregnant in April 1985 and soon thereafter had two severe relapses requiring up to 12 consecutive daily episodes of plasmapheresis to control her symptoms. Severe exacerbations recurred in the fifth and sixth months of gestation and were treated successfully with plasmapheresis. At 28 weeks of gestation, cramping lower abdominal pain simulating premature labor occurred without purpura. This spontaneously subsided during observation. At 37 weeks of gestation, cesarean section was performed to avoid the potential difficulty of differentiating relapse of Henoch-Schönlein purpura from other intra-abdominal conditions. A healthy female infant weighing 2700 gm was delivered. The placenta showed no vasculitis or infarction Despite persistent macroscopic hematuria, her blood pressure and renal function remained normal throughout the pregnancy.

The effect of pregnancy on the course of Henoch-Schönlein purpura remains unclear, and the treatment of relapses during pregnancy is unknown. Of the two

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reported cases, Plauche[1] first described a 19-year-old nulliparous woman who became pregnant 6 months after an episode of purpura, hematuria, and abdominal pain. Hypertension, azotemia, and worsening proteinuria necessitated cesarean section at 30 weeks of gestation with delivery of a viable infant. There was no recurrence of Henoch-Schönlein purpura during the pregnancy. McCoy[2] reported a 21-year-old nulliparous woman with purpura, fever, arthralgia, and abdominal pain at 17 weeks of gestation. The symptoms remitted in 2 weeks without any therapy and the rest of her pregnancy was uneventful. A viable infant was delivered vaginally at term. No information on changes in the placenta was available on either patient.

The patient reported herein appears to have a more severe form of Henoch-Schönlein purpura. Relapses became more frequent and severe during pregnancy and were treated with multiple episodes of plasmapheresis without adverse effect. Similarly, plasmapheresis has been used in the treatment of myasthenia gravis and Rh immune diseases during pregnancy without untoward effect. Steroids were not used. This procedure may be useful for symptomatic treatment of Henoch-Schönlein purpura in pregnancy.

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Theca-lutein ovarian cysts associated with homozygous α-thalassemia

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We present a case of theca-lutein cysts associated with homozygous α-thalassemia. Theca-lutein cysts have been associated with various types of nonimmune hydrops fetalis but have not been reported previously in association with homozygous α-thalassemia. (Am J Obstet Gynecol 1987;157:912-4.)

Key words: Thalassemia, theca-lutein cysts

Theca-lutein cysts of the ovary are most commonly associated with hydatidiform moles and choriocarcinoma. These cysts have been reported less commonly as a finding in nonimmune hydrops fetalis.[1] It is believed that theca-lutein cysts associated with nonimmune hydrops fetalis are caused by the highly elevated levels of human chorionic gonadotropin (hCG) produced by the enlarged hydropic placenta. The causes of nonimmune hydrops fetalis are many; more than 70 causes have been identified, including homozygous α-thalassemia.[2] We describe a patient with nonimmune hydrops fetalis caused by homozygous α-thalassemia and accompanied by large, bilateral theca-lutein cysts. This findings, first noted ultrasonographically during the third trimester of pregnancy, added a confusing dimension to our patient's clinical condition and care.

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Case report

A 28-year-old Vietnamese woman, gravida 1, para 0, was admitted to the Kaiser Permanente Medical Center, Santa Clara, California, at 30 weeks' gestation with clinical signs of preeclampsia. Physical examination showed blood pressure of 130/90 mm Hg and pretibial edema (2+). The uterus measured 34 cm in height. A rapid increase in fundal size had been noted over the previous 2 weeks. The cervix was closed with the vertex unengaged. Laboratory findings included a uric acid level of 11.0 mg/dl. All other values, including those for serum creatinine, blood urea nitrogen, serum glutamic oxaloacetic transaminase, complete blood count, platelet count, and glucose tolerance, were normal.

Ultrasound examination showed multiple abnormal findings. Polyhydramnios and a markedly enlarged placenta were present. Fetal findings included cardiomegaly, pericardial effusion, and hepatomegaly. No ascites or scalp edema was seen. Ultrasound scan also revealed large bilateral adnexal cystic masses measuring 5 by 7 cm each with alternating areas of cystic and solid material. Placental size and ultrasonographic findings induced us to search for a possible source of intra-

uterine infection or other cause of possible hydrops fetalis. Blood type was B, Rh-positive. Results of a VDRL test were nonreactive. Rubella and toxoplasmosis titers were negative. Cytomegalovirus titers were positive at 1:2169 dilutions and a herpes simplex titer was also positive at 1:1600 dilutions. Hematocrit was 38%. Mean corpuscular hemoglobin volume was noted to be 71 μm^3. The husband's complete blood count showed a mean corpuscular volume of 68.3 μm^3 (normal range >80 μm^3). The suspected diagnosis at that time was fetal homozygous α-thalassemia.

The patient's hospital course changed quickly with rapidly increasing polyhydramnios. Serial ultrasound scans done after admission to the hospital showed worsening fetal cardiomegaly. A nonstress test of the fetus showed an ominous pattern; that is, loss of short- and long-term variability and late decelerations were seen during a contraction stress test.

Because of the bilateral ovarian cysts, it was decided to perform an exploratory operation to rule out ovarian malignancy; this was done before death in utero occurred. A cesarean section was done to deliver the baby and to explore the abdominal cavity.

At operation, a 1290 gm infant was delivered and died in the delivery room. Bilateral theca-lutein ovarian cysts measuring 6 by 7 cm were found at operation. Biopsy of the left ovary showed typical changes in the theca-lutein cysts. The ovaries were then carefully returned to the abdominal cavity so as not to twist or rupture them. The placenta weighed 1600 gm.

The patient had an uneventful recovery from operation. Umbilical cord hemoglobin was evaluated (East Bay Cord Blood Screening Program for Hemoglobinopathies, Alta Bates Hospital, Berkeley/Children's Hospital, Oakland, California) and showed only Bart's hemoglobin, consistent with homozygous α-thalassemia. The β-subunit of hCG of the mother was studied serially in an attempt to explain the presence of theca-lutein cysts. The initial titer of the β-subunit of hCG was 1,120,000 mIU/ml. This level fell to 83.8 mIU/ml 15 days after operation and to <3 mIU/ml 5 weeks after delivery. No ovarian enlargement was palpable at examination 6 weeks after delivery. Ultrasound examination at this time confirmed that the ovaries were of normal size.

Comment

Theca-lutein cysts are usually found in association with hydatidiform moles or choriocarcinoma, suggesting that elevated secretion of trophoblastic hCG is responsible. Thecal luteinization and cyst formation may be caused by increased cystic atresis or by a heightened ovarian response to normal or elevated hCG levels. A correlation has also been demonstrated between elevated serum levels of hCG and hydrops fetalis. In such cases, the placentas are greatly enlarged, probably representing a source of increased hCG secretion.

A variety of conditions may give rise to hydrops fetalis, including Rh isoimmunization (a well-known cause), twin-twin transfusion syndrome, and many types of nonimmune hydrops, frequently idiopathic. The most frequent cause of hydrops fetalis in the Southeast Asian population is now recognized to be homozygous α-thalassemia or Bart's hemoglobinopathy. This condition is due to deficient α-globulin chain synthesis and is inherited as an autosomal recessive trait. Because of its high oxygen affinity, Barty's hemoglobin is useless as an oxygen carrier; the fetus therefore develops hydrops fetalis as a result of high-output cardiac failure. Homozygous α-thalassemia is incompatible with extrauterine life.

Our patient's pregnancy showed typical signs of Bart's hemoglobinopathy, that is, presence of mild microcytic anemia in the mother (low mean corpuscular volume unresponsive to iron therapy). Hydramnios and pregnancy-induced hypertension also commonly develop late in the second trimester or early in the third trimester. Intrauterine death from high-output cardiac failure is usually seen. The father, who was Asian, had similar blood test results, which showed mild anemia and a low mean corpuscular volume. Affected fetuses, as in this case, typically have cardiomegaly, frequently have ascites, and sometimes have pericardial effusion and hepatosplenomegaly caused by extramedullary hematopoiesis.

Since the α-thalassemia heterozygote produces no recognizable phenotype, detection requires careful screening, especially of indices in red blood cell morphologic analysis. Patients with a low mean corpuscular volume should be checked for iron-deficiency anemia and, if this is absent, hemoglobin electrophoresis should be performed. Normal hemoglobin electrophoresis results, normal iron stores, and low mean corpuscular volume are consistent with the α-thalassemia trait. Partner screening can then be done to evaluate the risk that offspring will have Bart's hemoglobinopathy of homozygous α-thalassemia. Identification of the trait allows genetic counseling of the couple as well as screening of their children at risk. This strategy is of importance in areas of the United States that have large Asian populations and should be part of the genetic and obstetric treatment plans of physicians practicing in these areas.

We have reported the association of large theca-lutein cysts and high levels of the β-subunit of hCG in a woman carrying a child with Bart's hemoglobinopathy. Two bilateral cystic ovarian structures shown ultrasonographically led to surgical exploration in the patient, who had a poor fetal prognosis. The nature of the ovarian cysts was recognized at operation and the cysts were not excised. This is essential because excision of these large ovarian tumors can result in loss of reproductive potential for the patient if the cysts are not recognized as being benign theca-lutein cysts. This pos-

sibility must be considered when any abdominal operation is done in a pregnant woman in order to avoid removing normal ovaries.

The association of theca-lutein cysts with hydrops fetalis is an uncommon finding. We report it here because, when hydrops fetalis is recognized in association with bilateral ovarian cysts, the association is most likely due to large theca-lutein cysts, and conservative management is indicated. The size of the placenta, as well as serum hCG levels, may also help diagnose this condition so that surgical exploration may be avoided.

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Racial differences in survival of women with endometrial cancer

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The hypothesis that white women with endometrial cancer survive longer than black women with this disease was evaluated in a retrospective analysis of a population-based, prospective cohort study. All female residents of metropolitan Atlanta with a first diagnosis of primary endometrial cancer from 1978 through 1982 were eligible for inclusion. The 628 white and 105 black women were followed up through June 1984 to determine survivorship. Race was evaluated as a prognostic factor with univariate, multivariate, and excess death rate analyses. Overall, an estimated 89.2% of whites and 61.6% of blacks survived 3 years from the time of diagnosis. Although black women tended to have more advanced disease at the time of diagnosis and a higher proportion of undifferentiated malignancies, the racial difference in survival persisted after adjustment for these factors. When initial therapy was considered, race remained a significant prognostic determinant among women who did not receive radiation therapy. (AM J OBSTET GYNECOL 1987;157:914-23.)

Key words: Survival, race, endometrial neoplasms, prognosis, mortality

Analyses of data collected by the National Cancer Institute concerning women diagnosed with cancer of the uterine corpus have repeatedly demonstrated inferior survival experiences for black women.[1-4] For patients diagnosed between 1973 and 1979, the 5-year relative survival rate for blacks (56%) was lower than that for Hispanics (87%) and American Indians (68%), which suggested that the effect was not entirely attributable to socioeconomic status.[3]

The conclusions reached in these surveys were limited in several respects. First, no adjustment was made for racial disparities in the distributions of histologic types. Second, no adjustment was made for possible racial differences in the distributions of histologic grades. Third, the broad categories used to characterize the extent of disease (e.g., localized versus others) may not have provided adequate control for racial dissimilarities in the stage of disease at the time of diagnosis. Finally, none of the analyses incorporated information on the types of treatments that were administered.

The purpose of the present investigation was to evaluate racial differences in survival of women with cancer of the endometrium. Analyses were adjusted for the effects of other prognostic factors, including age, marital status, socioeconomic status, histologic grade, stage of disease, and type of initial treatment.

Methods

The data for this study were collected by the Georgia Center for Cancer Statistics, an affiliate of the Surveillance, Epidemiology, and End Results Program. This registry is responsible for obtaining information on all

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incidences of cancer among residents of metropolitan Atlanta.[5]

Eligibility for inclusion in this study was limited to black or white female residents of the catchment area with an endometrial cancer diagnosed between Jan. 1, 1978 and Dec. 31, 1982. This accrual period was chosen because the most detailed information on the extent of disease was collected during those 5 years. The study population included 628 white women and 105 black women. The most common histologic type was adenocarcinoma (whites, 85.0%; blacks, 81.9%), followed by adenosquamous carcinoma (whites, 5.4%; blacks, 15.2%).

The primary method of case identification was the review of records at each of 37 hospitals and clinics in and around metropolitan Atlanta. Of the white women with endometrial cancer, 99.7% were identified from hospitalizations compared with 100% among black women. For both races, less than 0.1% of cases were registered from death certificates, which indicates that virtually all of the study population had a disease that was recognized clinically before the time of death.

Data were collected by a staff of trained medical record abstractors. Records were not abstracted until at least 4 months after the time of diagnosis to allow an assessment of the first course of medical treatment. Information about demographic, clinical, morphologic, and therapeutic variables was transcribed onto standard data collection forms, edited for completeness and internal consistency, coded following the guidelines of the Surveillance, Epidemiology, and End Results Program,[6] and entered into a computerized data base.

The demographic factors considered in this investigation were race (black versus white), age (years), marital status (married versus unmarried), and county of residence (Fulton county versus all others). The county of residence was used as an indicator of socioeconomic status, since Fulton County includes twice the percentage of persons living below the poverty level as found in the surrounding four suburban counties.[7] A more direct adjustment for socioeconomic status was not possible since data on income and education were not collected by the registry.

The tumor characteristics included the following: histologic grade of tumor (I = highly differentiated, II = moderately differentiated, and III = undifferentiated), depth of invasion (four ordinal categories; 1 = endometrium only, 2 = inner half of myometrium, 3 = outer half of myometrium, and 4 = invasion of serosa or extension into parametrium), evidence of cervical extension (present versus absent), and evidence of extrauterine involvement (present versus absent). Cervical extension was determined from the pathology reports when available. If a pathology report was unavailable, the clinical evaluation was used to characterize cervical extension (3%). Extrauterine involvement was defined as having at least one of the following present: direct extension of tumor beyond the uterus and parametrium, regional lymph node involvement, lymph node involvement outside of the pelvis, or distant site involvement. Extrauterine involvement was determined from the pathology reports or clinical evaluation.

Information about the first course of medical treatment included the following: surgical intervention (done or not done), type of surgical procedure performed, and radiation, hormone, and chemotherapy (each done or not done). In the present context, radiation therapy was defined as radiation treatment (preoperative and/or postoperative), with or without hormone and/or chemotherapy. Other therapy was defined as receiving hormone and/or chemotherapy.

Patients were followed up for vital status through the first half of 1984. The primary sources of information about survival were hospital records, which were supplemented by the records of private physicians and the quarterly review of computerized death certificates for the State of Georgia. Of the subjects who were known to be alive before 1982, 535 (97.3%) of the white women and 57 (95.0%) of the black women had a subsequent determination of vital status, which indicated small losses to follow-up.

The relationship between race and each of the other suspected prognostic factors was assessed by means of point estimates of the odds ratios and corresponding large sample approximate 95% confidence limits.[8] With death from cancer as the outcome, a univariate survival analysis was performed separately for each suspected prognostic factor by means of the Kaplan-Meier product limit estimator.[9] A generalized Wilcoxon test[10] was used to assess the statistical significance of differences between pairs of survival curves. With death from cancer as the outcome, the Cox proportional hazards regression model[11] was also used to evaluate the prognostic effect of race, with simultaneous adjustment for the effects of other prognostic factors.

To control for racial differences in expected deaths and avoid any possible bias from differential misclassification of causes of death, an excess death rate analysis was performed. For each racial group, the observed number of deaths from all causes was compared with the number of deaths expected from the woman-years of follow-up and the corresponding age, race, and sex-specific mortality rates for Georgia.[12] The excess death rate for each racial group was calculated as the difference between the observed and expected numbers of deaths per 1000 woman-years of follow-up.[13] A test statistic with a distribution that approximated a standardized normal deviate was used to assess the statistical significance of racial differences in excess death rates.[13]

Table I. Summary of demographic and tumor characteristics by race

Characteristic	*White women (n = 628)*	*Black women (n = 105)*	*Odds ratio**	
			Point estimate	*95% confidence limits*
Demographic				
Age at diagnosis (>60 yr)	345 (55)†	75 (71)†	2.05	(1.31, 3.22)
Marital status (unmarried)	239 (38)	71 (69)	3.56	(2.28, 5.56)
Place of residence (Fulton county)	227 (36)	75 (71)	4.42	(2.81, 6.94)
Tumor				
Histologic grade (III)	112 (21)	32 (37)	2.18	(1.35, 3.54)
Depth of invasion (>1/2 myometrium)	81 (15)	15 (19)	1.29	(0.70, 2.37)
Cervical extension (present)	64 (10)	26 (26)	2.97	(1.77, 4.97)
Extrauterine involvement (present)	56 (9)	19 (18)	2.26	(1.28, 3.97)

*Odds of index category among black women divided by odds of index category among white women.
†Numbers in parentheses are percentages of women with known values who were categorized into the index level.

Table II. Summary of treatment information by disease prognosis and race for women 85 years of age and younger

*Disease prognosis**	*Type of treatment received*	*White women*		*Black women*	
Favorable	No surgery	68 (15)†		11 (22)†	
	No other therapy		51 (75)‡		9 (82)‡
	Radiation therapy		17 (25)		2 (18)
	Other therapy		0 (0)		0 (0)
	Surgery	379 (85)		38 (78)	
	No adjuvant therapy		250 (66)		19 (50)
	Adjuvant radiation therapy		126 (33)		19 (50)
	Other adjuvant therapy		3 (1)		0 (0)
Unfavorable	No surgery	43 (26)		22 (41)	
	No other therapy		19 (44)		7 (32)
	Radiation therapy		18 (42)		12 (55)
	Other therapy		6 (14)		3 (12)
	Surgery	122 (74)		31 (58)	
	No adjuvant therapy		31 (25)		12 (39)
	Adjuvant radiation therapy		86 (71)		16 (52)
	Other adjuvant therapy		5 (4)		3 (9)

*Favorable = histologic grade I or II, no cervical extension, and no extrauterine involvement; unfavorable = all others.
†Percentage of women in category defined by disease prognosis and race.
‡Percentage of women in category defined by disease prognosis, race, and type of treatment.

Results

Relationship between race and other suspected prognostic factors

Demographic and tumor characteristics. The racial differences in demographic and tumor characteristics within the study population are summarized in Table I. The mean age ± standard deviation for black women was 64.1 ± 12.4 years (range: 26 to 89 years) compared with 62.2 ± 10.6 years (range: 25 to 97 years) for white women. The black women with endometrial cancer were significantly more likely to be unmarried and live in Fulton County.

Analysis of tumor characteristics indicated that black women had higher percentages of cervical and extrauterine involvement. Among women with recorded histologic grades (83%), the black women tended to have higher grades (I, 21%; II, 42%; and III, 37%) than those of white women (46%, 33%, 21%, respectively). Among women with depth of myometrial invasion information (84%), there was no racial difference in the percentage of women with invasion of the outer half of the myometrium or beyond.

Each of the demographic and tumor characteristics had less than 2% missing information except for histologic grade (17%) and depth of myometrial invasion (16%). There was no racial difference in the percentage of missing information for histologic grade (white, 16%; black, 18%, p = 0.67). The percentage of missing information for depth of myometrial invasion was greater (p = 0.02) for blacks (24%) than for whites (15%).

Distribution of treatments. Of the women with endo-

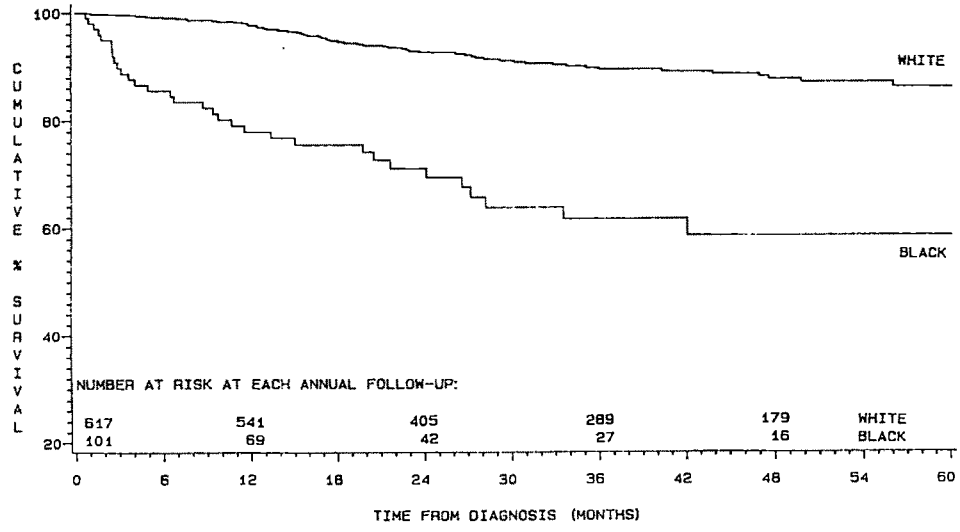


Fig. 1. Survival of women with endometrial cancer by race.

metrial cancer, 581 (79%) had surgery and 300 (41%) had radiation therapy. Since treatment could have been influenced by demographic and tumor characteristics, the distribution of treatments is presented in Table II by prognostic group and race for women 85 years of age and younger. Women with no cervical extension and no extrauterine involvement and a histologic grade of I or II were considered to have a favorable prognosis (i.e., Stage I disease with a differentiated neoplasm). All other women were considered to have unfavorable prognosis (i.e., Stages II through IV and/or an undifferentiated malignancy). For women without a recorded histologic grade, disease prognosis was determined by the stage of disease. For women less than or equal to 85 years of age at the time of diagnosis, 30% were classified as having unfavorable disease (white, 27%; black, 52%).

As indicated in Table II, the white women had significantly more surgery compared with black women (Mantel-Haenszel $\chi^2 = 5.51$, df = 1, p = 0.02). For women with favorable disease, 85% of the whites and 78% of the blacks had surgery. Within the unfavorable disease group, surgery was performed on 74% of the whites compared with 58% of the blacks. The racial difference in the percentage of women who received radiation therapy in addition to surgery was not uniform across the prognostic categories (test for homogeneity: $\chi^2 = 7.91$, df = 1, p = 0.005). As indicated in Table II, the blacks with favorable prognoses received more adjuvant radiation treatment compared with whites (50% for blacks and 33% for whites). Within the unfavorable category, 71% of white women received adjuvant radiation therapy compared with 52% among black women.

Univariate survival analysis. The relationship between race and survival in women with endometrial cancer is depicted in Fig. 1. Women older than 85 years of age at the time of diagnosis (10 whites and two blacks) and/or women with the date of diagnosis equal to the date of last follow-up (three whites and two blacks) were not included in any univariate survival analysis. The median follow-up time for the 617 white women was 34.1 months (range: <1 to 76.3 months) compared with a median follow-up time for 101 black women of 20.5 months (range: <1 to 77.1 months). For deaths caused by endometrial cancer, the product limit survival curve for white women was significantly ($p < 0.001$) higher than the corresponding survival curve for black women. For instance, 36 months after diagnosis, the cumulative percentage of survivors was 89.2% among whites but only 61.6% among blacks.

The univariate survival analyses of other demographic, treatment, and tumor characteristics are summarized in Table III. Women less than or equal to 60 years of age at the time of diagnosis, married women, and those residing outside of Fulton County had significant ($p < 0.005$) survival advantages.

All of the tumor and treatment characteristics except adjuvant radiation therapy were univariate predictors of survival ($p < 0.001$). Survivorship was associated with low histologic grade (I or II), depth of invasion limited to the endometrium or inner half of the myometrium, the absence of either cervical or extrauterine involvement, and having surgery performed.

Multivariate survival analysis

Demographic and tumor characteristics. The demographic and tumor characteristics were analyzed together to determine which variables were independent predictors of survival. The variables analyzed were race (1 = black), age at diagnosis (years), marital status

Table III. Summary of univariate survival analyses

Characteristic	*Category*	*n*	*Cumulative % survivors at 36 mo*	χ^2	*p value*
Demographic					
Race	White	617	89.2	67.2	<0.001
	Black	101	61.6		
Age at diagnosis	≤60 yr	311	93.8	28.9	<0.001
	>60 yr	407	79.0		
Marital status	Married	414	91.0	26.7	<0.001
	Unmarried	297	77.0		
County of residence	All others	425	88.4	8.0	0.005
	Fulton	293	81.4		
Tumor					
Histologic grade	I or II	458	92.4	69.1	<0.001
	III	141	65.1		
Depth of invasion	≤1/2 myomet	512	90.9	33.7	<0.001
	>1/2 myomet	93	70.1		
Cervical extension	Absent	618	89.1	55.6	<0.001
	Present	89	61.1		
Extrauterine involvement	Absent	646	89.8	168.8	<0.001
	Present	72	42.4		
Treatment					
Surgery	Yes	574	88.2	20.5	<0.001
	No	144	75.3		
Adjuvant radiation	Not received	326	91.7	1.7	0.19
	Received	248	83.7		

*Comparison of survival curves by the generalized Wilcoxon test.

Table IV. Summary of multivariate survival analyses

	*Model 1 (n = 588)**			*Model 2 (n = 588)†*		
Characteristic	*Regression coefficient*	*Standard error*	*p value*	*Regression coefficient*	*Standard error*	*p value*
Demographic						
Race	1.29	0.267	<0.001	2.09	0.393	<0.001
Age at diagnosis	0.03	0.016	0.035	0.03	0.016	0.073
Marital status	0.62	0.279	0.024	0.48	0.287	0.093
County of residence	−0.18	0.268	NS‡	−0.13	0.272	NS
Tumor						
Histology						
Grade II	0.58	0.412	NS	0.64	0.412	NS
Grade III	1.48	0.398	<0.001	1.47	0.402	<0.001
Cervical extension	0.33	0.307	NS	0.35	0.313	NS
Extrauterine involvement	1.75	0.305	<0.001	1.66	0.315	<0.001
Treatment						
Surgery	—	—	—	0.49	0.277	0.083
Radiation	—	—	—	0.48	0.322	NS
Interaction						
Race by radiation therapy	—	—	—	−1.54	0.533	0.004

*Includes demographic and tumor characteristics only.
†Includes demographic, tumor, and treatment characteristics.
‡NS = not statistically significant (p > 0.10).

(1 = unmarried), county of residence (1 = Fulton), histologic grade (characterized by two indicator variables: grade I = 0,0; grade II = 1,0; grade III = 0,1), depth of myometrial penetration (four ordinal categories), cervical extension (1 = present), and extrauterine involvement (1 = present).

Before demographic and tumor characteristics were tested for statistical significance as independent predictors of survival, possible interactions between race and each of the other individual characteristics were examined by means of the Cox proportional hazards regression model. None of the interaction terms was significant (p > 0.20).

In recognition of the high percentage of women with missing data (30%) on either histologic grade or depth of myometrial invasion and the association between his-

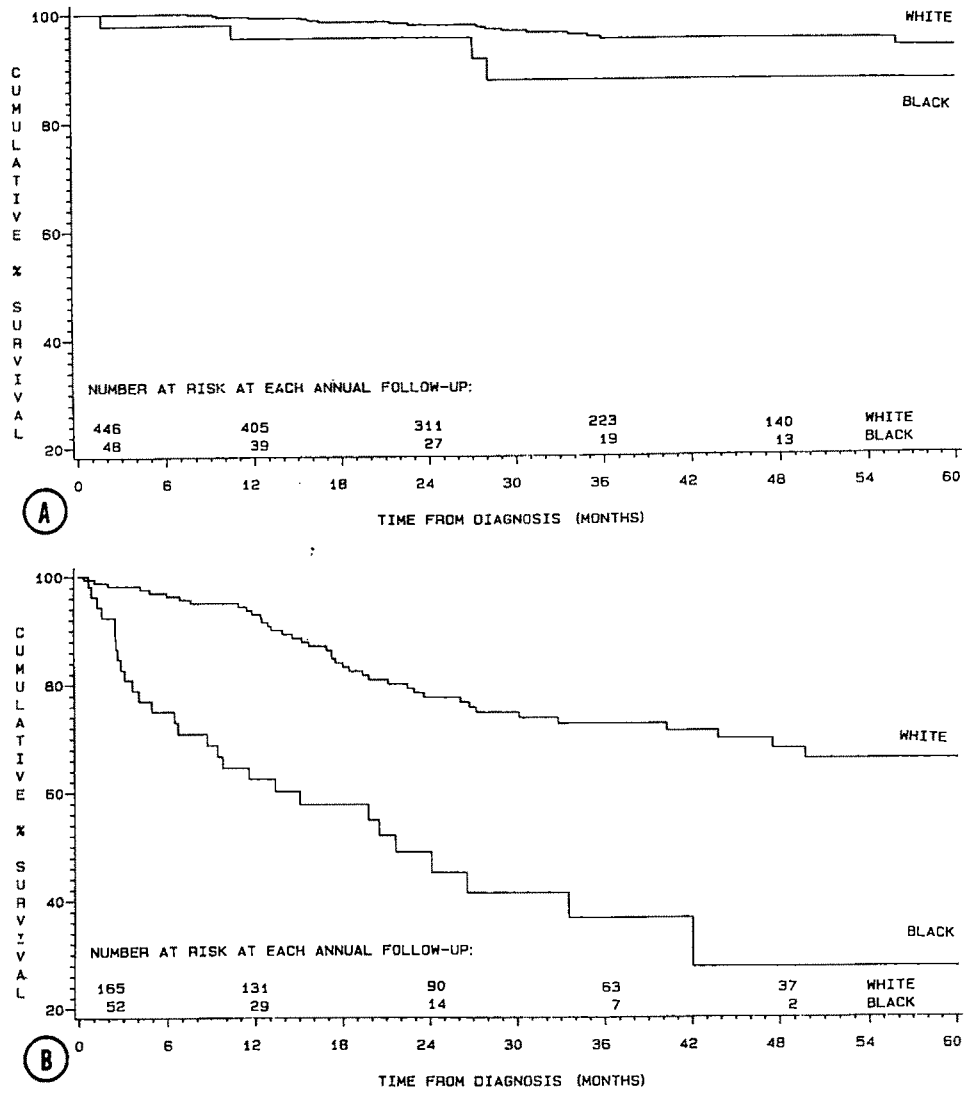


Fig. 2. A, Survival of women with favorable endometrial cancer by race. **B,** Survival of women with unfavorable endometrial cancer by race.

tologic grade and depth of invasion (test for independence: $\chi^2 = 42.8$, df = 4, $p < 0.001$), the Cox proportional hazards regression model was estimated separately with and without histologic grade or depth of myometrial invasion as predictors of survival. The total number of women less than or equal to 85 years of age at the time of diagnosis with complete information on all demographic and tumor characteristics except for histologic grade and depth of myometrial invasion was 702 (97%).

For these 702 women, the estimated hazard rate ratio (hazard rate of a black woman divided by the hazard rate of a white woman) unadjusted for the other demographic and tumor characteristics was 4.64, with 95% confidence limits of 2.98 and 7.20. In other words, the unadjusted rate of death among black women was elevated more than fourfold over the death rate for whites. Race was a significant ($p < 0.001$) predictor of survival after adjustment for the other demographic and tumor characteristics. The estimated hazard rate ratio after adjustment for age at diagnosis, marital status, county of residence, cervical extension, and extrauterine involvement was 3.57, with 95% confidence limits of 2.22 and 5.72.

The estimated regression model that used the demographic and tumor characteristics including histologic grade is presented as model 1 in Table IV. The total number of women less than or equal to 85 years of age with complete information was 588 (82%). The estimated unadjusted hazard rate ratio (blacks compared with whites) of the 588 women was 5.25, with 95% confidence limits of 3.33 and 8.49. In model 1, race was a significant ($p < 0.001$) predictor of survival after adjustment for the other demographic and tumor

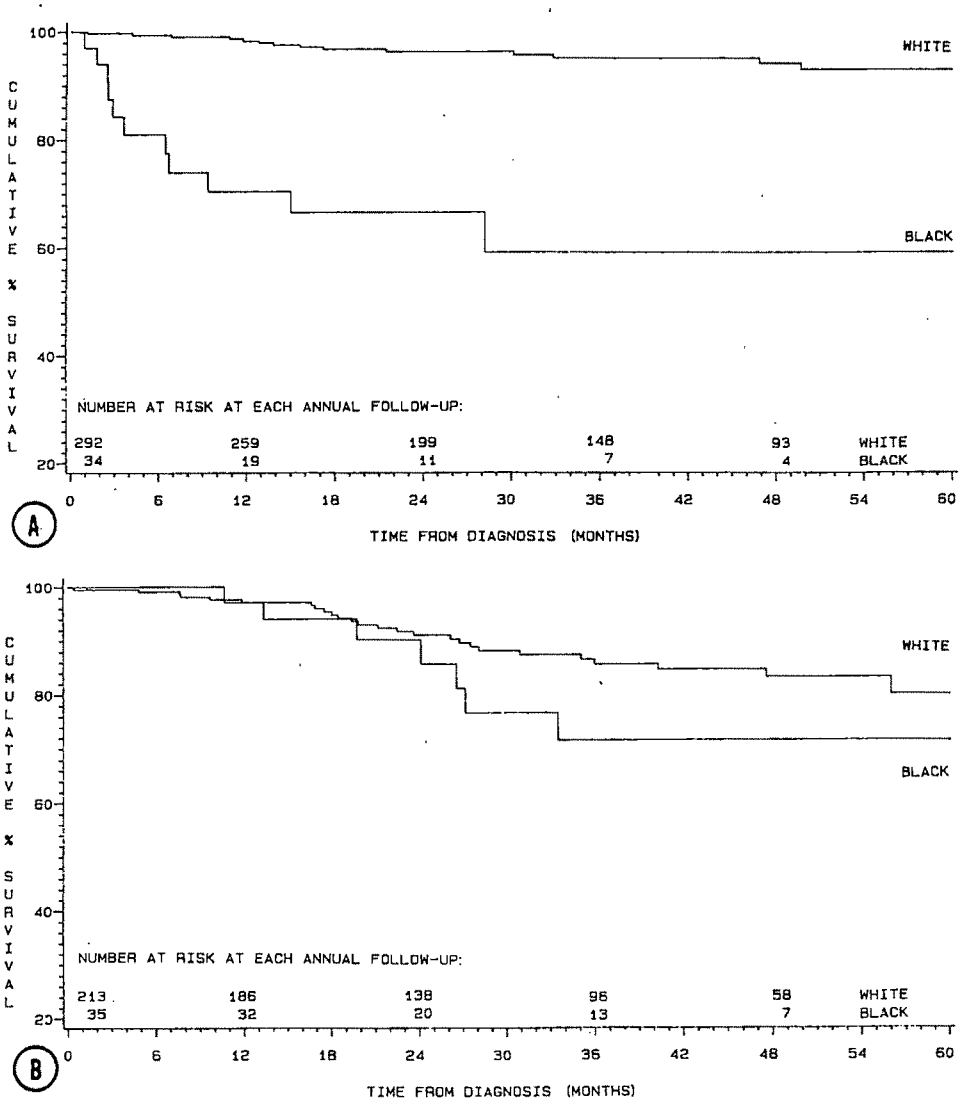


Fig. 3. A, Survival of women with endometrial cancer by race for patients who did not receive adjuvant radiation therapy. **B,** Survival of women with endometrial cancer by race for patients who received adjuvant radiation therapy.

characteristics. The estimated hazard rate ratio after adjustment for age at diagnosis, marital status, county of residence, histologic grade, cervical extension, and extrauterine involvement was 3.62, with 95% confidence limits of 2.15 and 6.12. Thus the estimated prognostic effect of race was not altered by the inclusion of histologic grade into the regression model. The inclusion of depth of myometrial invasion instead of histologic grade along with the demographic and tumor characteristics of model 1 also did not alter the prognostic effect of race.

To characterize further the racial difference in survival found in the multivariate analysis, the race-specific survival curves for deaths caused by endometrial cancer are depicted in Fig. 2, *A*, for women with favorable prognoses and in Fig. 2, *B*, for women with unfavorable prognoses. Among women with favorable disease, the product limit survival curve was significantly higher for whites compared with blacks (generalized Wilcoxon test: $\chi^2 = 3.95$, df = 1, $p = 0.047$). An even greater racial difference in survival was detected among the women with unfavorable prognoses (generalized Wilcoxon test: $\chi^2 = 30.7$, df = 1, $p < 0.001$).

Inclusion of treatment factors. The analysis was extended to include information on treatment and the previously mentioned demographic and tumor characteristics. The potential prognostic treatment factors considered were surgical treatment (1 = not done) and radiation treatment (1 = done).

Before reconstruction of the regression models, possible interactions between race and each of the treatment factors were estimated. Survival was better for

Table V. Excess death rate during 3 years of follow-up

Category	n	No. of deaths		Woman-years of follow-up	Excess death rate†	Racial comparison	
		Observed	Expected*			Z test	p value
All women	710	126	28.7	1572	61.9		
White	612	80	23.4	1402	40.3	4.92	<0.001
Black	98	46	5.3	170	239.4		
Histologic grade							
I or II							
White	403	28	14.7	942	14.1	2.81	0.005
Black	51	15	2.7	95	130.1		
III							
White	110	38	4.8	230	144.5	2.83	0.005
Black	29	21	1.5	43	458.3		
Cervical extension							
Absent							
White	540	52	20.9	1268	24.6	4.10	<0.001
Black	71	30	4.1	133	195.3		
Present							
White	64	26	2.2	118	201.2	1.57	0.116
Black	24	14	1.1	33	392.0		
Extrauterine involvement							
Absent							
White	558	48	21.4	1317	20.2	3.96	<0.001
Black	81	30	4.8	158	158.7		
Present							
White	54	32	2.0	85	352.2	2.82	0.005
Black	17	16	0.5	11	1359.7		
Disease prognosis‡							
Favorable							
White	442	23	16.5	1056	6.2	2.24	0.025
Black	46	11	2.9	98	82.4		
Unfavorable							
White	164	57	6.8	333	150.9	3.65	<0.001
Black	51	35	2.3	69	476.9		
Treatment							
No adjuvant radiation							
White	288	24	10.4	678	20.1	3.52	<0.001
Black	33	17	1.5	47	328.4		
Adjuvant radiation							
White	213	31	8.0	481	47.8	1.89	0.059
Black	35	13	2.3	77	139.3		

*Based on age, race, and sex-specific death rates for all causes of death.
†((Observed deaths-expected deaths)/(woman-years of follow-up)) × 1000.
‡See Table II for definitions of favorable and unfavorable prognosis.

whites compared with blacks, regardless of whether surgery was performed. Among women who received surgery without adjuvant radiation therapy, whites had a significantly higher survival probability than did blacks (Fig. 3, *A*, generalized Wilcoxon test: $\chi^2 = 57.8$, df = 1, $p < 0.001$). There was no significant racial difference in survival among the women who received adjuvant radiation therapy (Fig. 3, *B*, generalized Wilcoxon test: $\chi^2 = 1.6$, df = 1, $p = 0.208$).

The estimated regression model that used the demographic, tumor, and treatment characteristics except for histologic grade indicated that race was a significant predictor of survival among women not receiving radiation treatment (estimated hazard rate ratio = 6.90, with 95% confidence limits of 3.41 and 14.0). Among women receiving radiation treatment, race was not a significant predictor of survival (estimated hazard rate ratio = 1.84, with 95% confidence limits of 0.72 and 4.75).

The estimated regression model that used the same demographic, tumor, and treatment characteristics, and histologic grade is presented as model 2 in Table IV. Again, race was a significant predictor of survival among women not receiving radiation treatment (estimated hazard rate ratio = 8.05, with 95% confidence limits of 3.73 and 17.4) but not among women receiving radiation treatment (estimated hazard rate ratio = 1.73, with 95% confidence limits of 0.60 and 4.98). Replacement of histologic grade by depth of myometrial invasion did not alter the prognostic effect of race, which indicated the absence of confounding by either histologic grade or depth of invasion.

Excess death rate analysis. The observed and expected number of deaths from all causes and the cor-

responding excess death rates from all causes during a 36-month follow-up period for women surviving at least 1 month are presented in Table V. For the entire study population, 61.9 more deaths were observed per 1000 woman-years of follow-up than would be expected in a population of Georgians with the same distribution of age, race, and sex.

When excess death rate analysis was performed separately for each racial group, the excess death rate for black women of 239.4/1000 woman-years was significantly greater ($p < 0.001$) than that of 40.3/1000 woman-years among white women. Stratification of the excess death rate by race and separate tumor characteristics revealed a significantly ($p < 0.005$) greater rate of excess deaths for black women with or without differentiated tumors, women with no cervical extension, and women with or without extrauterine involvement. The excess death rate for blacks also tended to be greater than that for whites among women with cervical extension; however this difference did not reach statistical significance ($p = 0.12$).

The effect of tumor characteristics on the racial difference in excess death rates was characterized further by the disease prognosis classification (favorable versus unfavorable). The excess death rates (per 1000 woman years) among women with favorable disease were 6.2 for whites and 82.4 for blacks. Among women with unfavorable prognoses, the excess death rates were 150.9 for whites and 476.6 for blacks. The estimated excess death rate for whites was significantly lower among women with favorable disease ($z = 2.24$, $p = 0.025$) and among women with unfavorable disease ($z = 3.65$, $p < 0.001$). The racial difference in excess death rates was significantly greater among the women with unfavorable prognoses compared with women with favorable disease (test for heterogeneity: $z = 2.61$, $p = 0.009$).

The racial difference in excess death rates of women with endometrial cancer by adjuvant radiation treatment indicated again that there was a greater racial difference in excess death rates for women who received no adjuvant radiation treatment compared with women who received adjuvant radiation treatment (test for homogeneity: $z = 2.16$, $p = 0.03$).

Comment

The most consistently reported prognostic factors for women with endometrial cancer are age,[14, 15] depth of myometrial invasion,[14-16] and histologic grade.[15, 16] In the present investigation, each of these variables was found to be a predictor of survival in both univariate and multivariate analyses. As noted elsewhere,[16] histologic grade is related to age, with a higher proportion of undifferentiated lesions in elderly women.

In addition to the established prognostic factors for endometrial cancer, the present work indicated that race was a statistically significant predictor of survival. The influence of race was strongest for women with undifferentiated malignancies and/or nonlocalized disease. Among women who did not receive radiation treatment, blacks had a worse survival experience than whites after adjustment for other demographic and tumor characteristics. In contrast, no racial difference in survival was observed among women who underwent radiation therapy.

There are several possible explanations for the present findings. First, it is possible that systematic errors in sample selection or data collection may have occurred. With regard to the sampling strategy, an attempt was made to identify all women with endometrial cancer from a large biracial metropolitan community. Although clinically occult neoplasms could have escaped detection, the small percentage (0.3%) of patients who were diagnosed only at autopsy implies that the incidence of clinically unrecognized endometrial cancer is low.

A greater potential loss of subjects from the present study was related to the absence of complete information on all variables of interest. Almost one third of the study population had missing data on either histologic grade or depth of myometrial invasion. To minimize the exclusion of subjects with missing data from the regression models, multivariate analyses were constructed with the inclusion of either histologic grade or myometrial invasion. Adjustment for the separate effects of these variables did not alter the prognostic significance of race. Since similar results were obtained regardless of which subjects were excluded, it does not appear that bias was introduced through missing information.

The findings of this investigation might have also been influenced by inaccuracies within the collected data. However, misdiagnoses are unlikely in the present context, since microscopic confirmation of disease was required for eligibility. The primary outcome of this study was death from endometrial cancer. It is possible that the assignments of causes of death differed systematically for white and black women with endometrial cancer. The excess death rate analysis, however, was based on a comparison of relative mortality rates and did not require specification of causes of deaths. Since the results of the excess death rate analysis were consistent with the other analyses, it is unlikely that the racial difference in survival was attributable to differential classification of causes of death. Although it might have been useful to analyze other outcomes, such as treatment failures or recurrence of disease, the registry does not collect information on these events.

Several additional characteristics of this investigation should be noted. First, the median lengths of follow-

up were relatively short: white women, 34.1 months and black women, 20.5 months. As a consequence, long-term prognosis could not be evaluated. Second, the type of available information on treatments was limited, and therefore the effects of factors such as the dose of radiation therapy could not be evaluated. Third, the collected data only pertained to treatments that were administered in the first 4 months after diagnosis.

Despite these methodologic constraints, the results of the present investigation indicated a strong racial difference in the survival of women with endometrial cancer. The magnitudes of racial differences in survival probabilities were substantial and therefore unlikely to be attributable to chance. The most reasonable explanations for the observed disparity in survival relate to racial differences in the clinical management of women with undifferentiated malignancies and/or nonlocalized disease. Among such women, whites had a greater frequency of surgery than blacks. The overall use of radiation therapy was also more common among whites than blacks with unfavorable grades and/or stages. Moreover, the racial difference in survival was greatly diminished among women who underwent radiation therapy.

The present findings emphasize the need for further research on factors that may influence the choice of therapy for women with endometrial cancer. In particular, subsequent investigations should include data on the presence of concurrent illnesses, patient noncompliance with recommended treatments, and complications of therapy. Until such data are collected, it is premature to speculate on whether black women with endometrial cancer receive optimal clinical management.

The authors express their appreciation to the staff of the Georgia Center for Cancer Statistics who collected, coded, edited, and processed these data, and to the hospitals and physicians who provided information on patients with endometrial cancer. Mr. Scott Clark helped perform the statistical analyses.

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Endometrial adenocarcinoma estrogen receptor content: Association of clinicopathologic features with immunohistochemical analysis compared with standard biochemical methods

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Endometrial adenocarcinomas from 96 patients were studied for their biochemical estrogen receptor content as well as immunohistochemical localization of estrogen receptor. A well-characterized specific monoclonal antihuman estrogen receptor antibody (H222 sp γ) as amplified by peroxidase/antiperoxidase techniques was used for immunohistologic localization. Immunohistochemical evaluation incorporated both the intensity and distribution of staining into a semiquantitative analysis (HSCORE). The total HSCORE was the sum of the relative contributions of the four histologic components: benign epithelium, malignant epithelium, stroma, and myometrium. These results were compared with clinicopathologic features of the tumors. Excellent sensitivity (91.5%) and specificity (93.1%) were observed for immunohistochemical analyses as compared with the biochemical analysis of estrogen receptor for these tissues. The cancer component HSCORE correlated better with grade than did the biochemical estrogen receptor determination. Correlation was also observed between surgical stage and estrogen receptor content. Decreasing biochemical estrogen receptor content predicted advanced surgical stage ($p = 0.0003$), as did the total HSCORE ($p = 0.003$); however, the HSCORE of the cancer component only did not correlate with advanced surgical stage ($p = 0.11$). Although immunohistochemical analyses predicted biologic differentiation better than did the biochemical technique, stage correlated better with biochemical estrogen receptor analysis and total HSCORE than did the cancer component HSCORE. The HSCORE of the cancer component may better predict biologic behavior and therefore identify cancers more likely to respond to hormonal therapy. (Am J Obstet Gynecol 1987;157:924-31.)

Key words: Estrogen receptor, monoclonal antibody, endometrial carcinoma

Hormonal manipulation is effective in the treatment of many neoplasms derived from steroid-dependent tissues. The ability of steroid receptor content to predict biologic behavior in patients with breast carcinoma has been well established.[1] The clinical value of steroid receptor analysis has not been as well established for endometrial carcinomas, although clinicopathologic associations with receptor content have been observed.[2, 3] Marked tissue heterogeneity seen in endometrial carcinoma specimens may confound interpretation of steroid receptor content derived from biochemical assays of whole tissue homogenates. Unlike nonneoplastic breast tissues, which contain only low levels of sex steroid receptor, uterine and endometrial tissues often contain appreciable amounts of receptor. The relative amount that the estrogen receptor content of myometrial, stromal, and benign glandular epithelial elements contribute to the total estrogen receptor of tissue homogenates of individual endometrial carcinoma specimens is not clear, but these benign elements could contribute significantly to measured receptor.[4, 5] In addition, endometrial carcinomas often show multiple patterns of differentiation and cell type within individual lesions, often within a single histologic section of that lesion.[5]

Concentrations of cytosolic estrogen and progesterone receptors from different regions of the normal uterus are not identical.[6] The current biochemical assays of estrogen receptor content cannot account for heterogeneity of tissue type and the contribution to the total estrogen receptor content of tumor tissue samples, because they are performed on whole tissue homoge-

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nates. Homogenation obscures the source and proportion of cells in heterogeneous populations.

The desire to develop reliable histochemical assays for estrogen and progesterone receptors stems principally from considerations of the potential significance of heterogeneity of tissue type and heterogeneity of tumor receptor content, as well as the need for relatively large amounts of tissue for the usual biochemical steroid receptor assay. Previous attempts to evaluate receptor content with fluorescent-conjugated steroids or antisteroid hormone antibodies have not been successful.[7, 8] The recent development of specific monoclonal antibodies against human estrogen receptors offers the opportunity for specific localization of estrogen receptor within various tissue components.[9, 10] We compared clinical and pathologic associations of biochemical and immunohistochemical estrogen receptor assays of endometrial carcinomas to determine the significance of the estrogen receptor contribution of noncancerous components.

Material and methods

Ninety-six specimens from patients with endometrial adenocarcinoma treated at Duke University Medical Center from January 1, 1980, to December 31, 1983 were submitted for histopathologic examination and analysis of estrogen receptor content by both biochemical and immunohistochemical techniques. All patients were surgically managed and tissue was obtained from the hysterectomy specimen. None of the patients received preoperative radiation. In addition to preoperative staging according to the International Federation of Gynecologists and Obstetricians guidelines, all patients were retrospectively surgically staged. Surgical staging consisted of total abdominal hysterectomy, pelvic and periaortic selective lymphadenectomies, and pelvic washings. Retroperitoneal lymph nodes were sampled in patients with grades 2 and 3 lesions, as well as all grade 1 lesions with deep myometrial invasion detected at the time of surgery. Evaluation of myometrial invasion was made on the basis of intraoperative sectioning of the uterus. Patients with Stage I, grade 1 lesions and only superficial myometrial invasion underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic cytologic examination.

Surgical stages were defined as follows: Stage I, disease confined to the uterus; Stage II, disease involving the cervix; Stage III, lymph node or pelvic metastases; and Stage IV, disease in upper abdomen or distant metastases. All grading of specimens was performed by three observers (K. S. M., Jr., D. B., and J. T. S.) on material submitted for estrogen receptor analyses.

Biochemical estrogen receptor analyses. Estrogen receptor content of the tissues was analyzed according to a modification of the methods described by Schrader et al.[11] Tissue was obtained immediately in the fresh state, washed in buffer (10 mmol/L Tris HCl, 5 mmol/L HEPES HCl, 1.5 mmol/L EDTA, 1.0 mmol/L thioglycerol, and 0.02% NaN_3, pH 7.4) at 4° C, quick frozen in liquid nitrogen, and maintained at −80° C in airtight liquid nitrogen capsules until assayed. The frozen tissue was pulverized in liquid nitrogen by a Spex freezer mill at 3/4 power with a stainless steel impeller for 15 seconds (five 3-second cycles). The tissue powder was homogenized at a sample:buffer volume of 1:6 at 4° C by a Polytron (setting 3) for 60 seconds (four 15-second cycles with 30-second cooling periods). Cytosol was prepared from the homogenate by centrifugation at 1000 × g for 10 minutes followed by 145,000 × g for 1 hour, both a 4° C. Endogenous unbound steroid was removed by 0.75% (w/v) Norit A in 0.0025% dextran. The supernatant protein content was analyzed by the method of Bradford[12] and adjusted to a protein content of 4 to 6 mg/ml. Sucrose density gradient analysis and multiconcentration dextran-coated charcoal analysis were performed as previously described.[13]

Immunohistochemical estrogen receptor analysis. Portions of the tissue specimens that had been fresh frozen in liquid nitrogen for biochemical assays were prepared as serial 4 to 6 μm cryostat sections mounted on poly-L-lysine–coated slides. The initial section was fixed in 3.7% formalin in 0.01 mmol/L phosphate buffered saline solution and stained with hematoxylin-eosin for tissue diagnosis and correlation with the presence of immunohistochemical staining. Histologic grade of the tumor was evaluated based on the cryostat section prepared for immunohistochemical receptor analyses (see below) with classification according to International Federation of Gynecologists and Obstetricians criteria. Assignment of tumor type and grade was confirmed by review of formalin-fixed, paraffin-embedded, hematoxylin-eosin–stained sections taken from the same specimens.

Monoclonal antibody H222 Sp γ developed against MCF-7 human breast cancer estrogen receptors by Dr. L. S. Miller of Abbott Laboratories was selected for this study. This monoclonal antibody has been shown to be specific for human estrogen receptors from human endometrium and human breast cancers by several criteria.[14] H222 Sp γ recognizes a stable and well-conserved determinant that is close to the steroid binding site. Peroxidase/antiperoxidase staining for immunohistochemical localization was performed as follows. Cryostat sections were placed in 3.7% formaldehyde–phosphate-buffered saline solution for 10 minutes. They were then transferred to a bath until formalin fixation had been completed for all sections in a given run. Slides were placed in cold absolute methanol at −15° to −20° C for 4 minutes, and then in cold acetone at −15° to −20° C for 1 minute. Afterward,

Table I. Stage versus grade

	Grade 1	*Grade 2*	*Grade 3*	*Total*
Stage I	29	30	6	65 (67.7%)
Stage II	4	1	4	9 (9.4%)
Stage III				
Nodes	6	2	5	13 (13.5%)
Pelvic metastases	1	4	2	7 (7.2%)
Stage IV		1	1	2 (2%)
Total	40 (41.6%)	38 (39.5%)	18 (18.7%)	

slides were washed in phosphate-buffered saline solution twice for 5 minutes each at ambient temperature. Sections were treated with 2% normal goat serum in phosphate-buffered saline solution for 15 minutes in a humidified chamber, to reduce the nonspecific binding of bridging antibody (see below). Sections were incubated with primary antibody (H222 Sp γ, 5 μg/ml; 33 pmol/ml in phosphate-buffered saline solution) for 30 minutes at ambient temperature. Negative control slides of adjacent cryostat sections from each of the same tissues were incubated with control antibody (normal nonimmunized rat serum in phosphate-buffered saline solution) in place of the primary antibody for 30 minutes. Control slides of estrogen receptor–rich MCF-7 human breast cancer cells were incubated with primary or control antibody. Slides were then washed in phosphate-buffered saline solution two times for 5 minutes each. Sections were incubated with bridging antibody (goat antirat immunoglobulin in phosphate-buffered saline solution) for 30 minutes. Slides were again washed in phosphate-buffered saline solution twice for 5 minutes each. Peroxidase-antiperoxidase complex (horseradish, peroxidase, and rat antihorseradish peroxidase in phosphate-buffered saline solution) was applied to the sections for 30 minutes, after which slides were washed in phosphate-buffered saline solution twice for 5 minutes each. The slides were then flooded with 8 mg diaminobenzidine/16 ml hydrogen peroxide in phosphate-buffered saline solution for 6 minutes in the dark. The solution had been prepared under minimal light and was used within 30 minutes. Sections were rinsed in gently running tap water for 5 minutes and dehydrated in serial alcohols to xylene; coverglasses were applied with Permount without counterstaining.

In the presence of hydrogen peroxide, the tissue-bound peroxidase converts the diaminobenzidine chromagen to an insoluble, brown reaction product that can be seen with a light microscope. Thus the minute quantity of estrogen receptor/monoclonal antibody complex is amplified through the use of the bridging antibody, peroxidase-antiperoxidase complex, and enzyme reaction products.

Scoring of assays. Biochemical assays were summarized as femtomoles of specific estrogen binding per milligram of tissue protein. The immunohistochemical localization was scored in a semiquantitative fashion incorporating both the intensity and distribution of specific staining. Evaluations were recorded for each observed tissue component (i.e., myometrium, stroma, benign epithelium, and malignant epithelium) as the percentage of cells in each of four intensity categories. The intensity of specific staining was characterized as not present (0), weak but detectable above control (1+), distinct (2+), and very strong (3+). For each observed tissue component, a summary value (HSCORE) was calculated that consisted of a sum of the percentages of positively stained cells multiplied by a weighted intensity of staining:

$$\text{HSCORE} = \sum_{i=0} P_i(i + 1)$$

where P_i is the percentage of stained cells in each intensity category and i is the intensity for i = 1,2,3. A total HSCORE for the tissue section was derived as the sum of the component HSCORE weighted by the fraction of each component observed in the tissue section, as follows:

Total HSCORE =
[(Cancer HSCORE × percent cancer)/100]
+ [(stromal HSCORE × percent stroma)/100]
+ [(myometrial HSCORE × percent myometrium)/100] +
[(benign epithelium HSCORE × percent benign epithelium)/100

Biochemical assays were compared with the total HSCORE as well as the individual component HSCORE values.

Sections were evaluated independently by two observers and were further reviewed by the initial observer. Intraobserver ($r = 0.98$, $p = 0.00001$) and interobserver differences for total HSCORE were resolved by consensus evaluation ($r = 0.99$, $p = 0.00001$ for primary observer versus consensus total HSCOREs).

Data management and analysis. Biochemical assay values and immunohistochemical assay values were coded separately in a blinded fashion and maintained

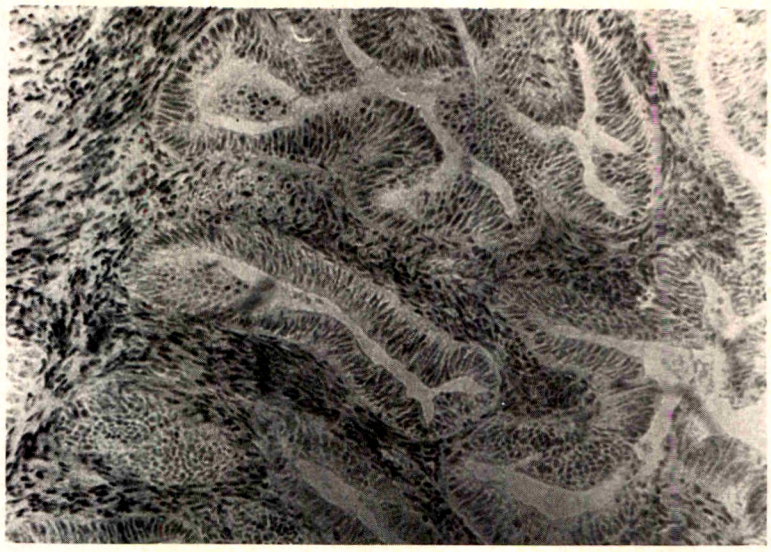


Fig. 1. Very well-differentiated adenocarcinoma of the endometrium shows marked myometrial staining for estrogen receptor. Nuclear staining is almost exclusively present.

as independent files in the Time-Oriented Record for Oncology system of the Duke Cancer Center database until the completion of each phase of the study. Record identification numbers were used to match samples for the analysis. Accuracies, sensitivities, specificities, and predictive values were determined. The correlations were analyzed with the Spearman rank test for ordinal data (stage, grade) or the Pearson correlation for continuous data (biochemical estrogen receptor, HSCORE). Significance of two-way tables was determined by χ^2 test or Fisher's exact test as appropriate. Differences in assay values between groups based on stage and grade were tested for significance by the Mann-Whitney U test. Distributions were plotted as the cumulative proportion of observations having a value less than or equal to the corresponding value on the abscissa (the cumulative distribution function).

Results

Ninety-six patients were evaluated completely. Forty patients (41.6%) had grade 1, 38 (39.5%) had grade 2, and 18 (18.8%) had grade 3 lesions. Sixty-five patients (67.7%) had surgical Stage I disease, nine (9.4%) had surgical Stage II disease, 13 (13.5%) had Stage III disease with pelvic metastases, seven (7.2%) had Stage III disease with lymph node metastasis, and two (2.0%) had Stage IV disease (Table I). Increasing surgical stage correlated with worsening tumor grade ($p = 0.016$, Spearman).

Immunohistochemical estrogen receptor analysis with monoclonal antibody H222 Sp γ characteristically showed nuclear localization of specific staining above control values in all tissue components. Critically, the amount of staining in the cancer component often diverged from that in the stromal or myometrial components (Figs. 1 and 2). Fig. 1 shows marked myometrial staining with only a small amount of epithelial localization. In contrast, Fig. 2 shows marked staining of the malignant epithelial component with little localization in surrounding myometrium or stroma. Fig. 2 also demonstrates a heterogeneous pattern of staining within the malignant epithelial tissue itself. Among stromal and myometrial elements, however, staining tended to be more homogeneous, and in normal epithelium staining was usually uniform.

The total HSCORE correlated strongly with the biochemical estrogen receptor assay. Threshold positivity (≥10 fmol/mg protein) was predicted by the total HSCORE (≥75) with a sensitivity of 92% and specificity of 93%. Total HSCORE correlated more closely with estrogen receptor ($r = 0.68$) than with the malignant epithelial ($r = 0.65$) and stromal or myometrial ($r = 0.35$) components.

When lesions were categorized by histologic grade, estrogen receptor localization by the immunohistochemical technique in the cancer component only was virtually absent in the majority (88% negative) of poorly differentiated (grade 3) cancers; it was present in most (92%) of the well-differentiated tumors (grade 1); values were intermediate in the group with intermediate differentiation (grade 2). In the latter group there appeared to be two, and possibly three, distinct populations of lesions identified by the HSCORE of the cancer component: negative, low positive, and strongly positive (Fig. 3).

Biochemical analysis of estrogen receptor content did not distinguish among populations of differing histologic grade as well as the HSCORE of malignant epi-

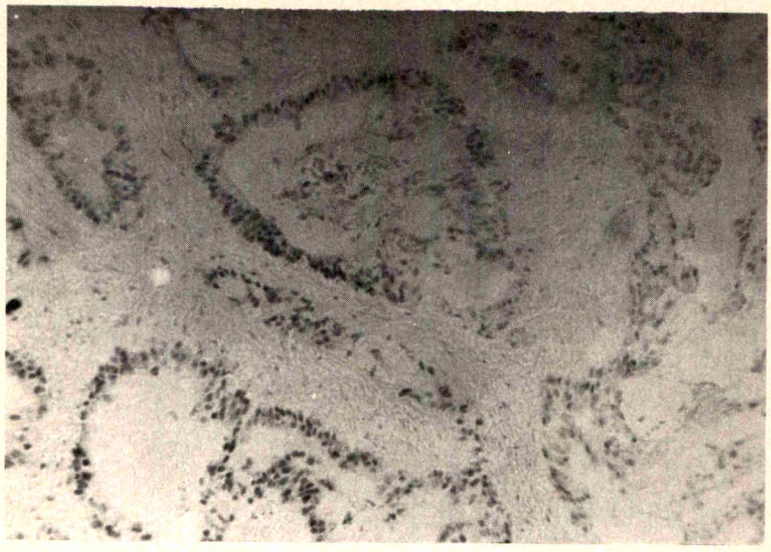


Fig. 2. Moderately differentiated adenocarcinoma of the endometrium. There is a weak myometrial staining relative to cancer component staining and heterogeneity of staining within the cancer component.

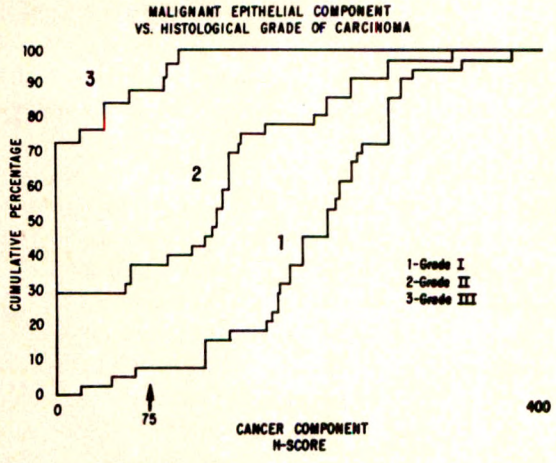


Fig. 3. Cumulative distribution function of cancer component HSCORE versus grade. *Arrow* denotes threshold value of 75. Of the grade 1 lesions, 8% are estrogen receptor–negative; 88% of grade 3 lesions are estrogen receptor–negative. Increments are from 40 to a total score of 400. 1 versus 2, $p = 0.000027$; 1 versus 3, $p < 0.00001$; 2 versus 3, $p < 0.00005$.

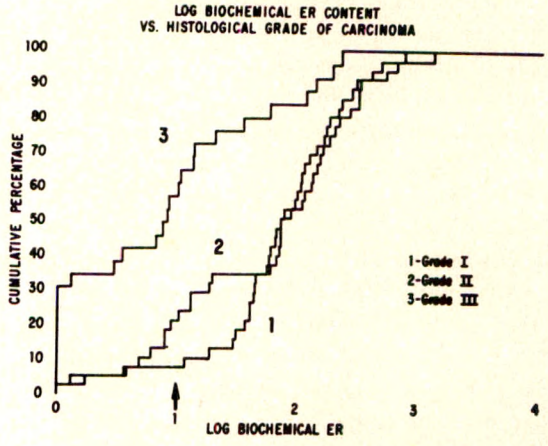


Fig. 4. Cumulative distribution function of log biochemical estrogen receptor *(ER)* versus grade. *Arrow* denotes threshold value of 10 fmol/mg protein. Of the grade 1 lesions, 8% are estrogen receptor–negative; 57% of grade 3 lesions are estrogen receptor–negative. 1 versus 2, $p = 0.18$; 1 versus 3, $p = 0.00002$; 2 versus 3, $p = 0.0004$.

thelial component did. The distribution of the biochemical estrogen receptor assay among grades 1 and 2 was indistinguishable, but these distributions were clearly distinct from that in grade 3 lesions (Fig. 4). The total HSCORE distribution was intermediate between the biochemical estrogen receptor and the cancer component HSCORE in discriminating among histologic grade. The cumulative distribution function demonstrates clear discrimination between each of the three grades (Fig. 5). However, the degree of separation was not as distinct as with the cancer component HSCOREs alone. There was a relatively equal estrogen receptor staining contribution of the stroma and myometrial elements in each histologic grade of the cancer.

Cancer component HSCOREs were not significantly associated with clinical or surgical stage (Fig. 6) as determined by comparison of intrauterine versus extrauterine disease ($p = 0.11$, Mann-Whitney). The distribution of total HSCORE between groups of Stages I

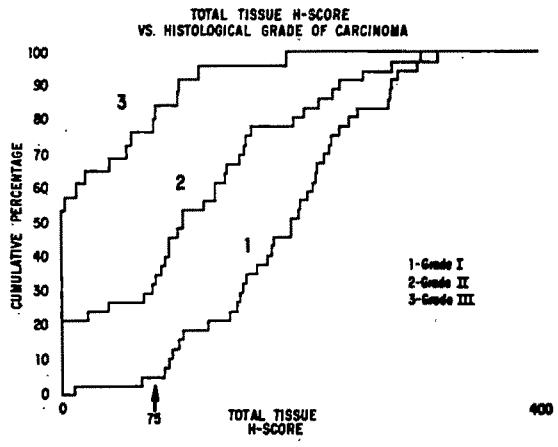


Fig. 5. Cumulative distribution function of total tissue HSCORE versus grade. *Arrow* denotes threshold value of 75. Of the grade 1 lesions, 5% are estrogen receptor–negative. Increments are from 40 to a total score of 400. 1 versus 2, p = 0.0008; 2 versus 3, <0.00001; 2 versus 3, p = 0.00006.

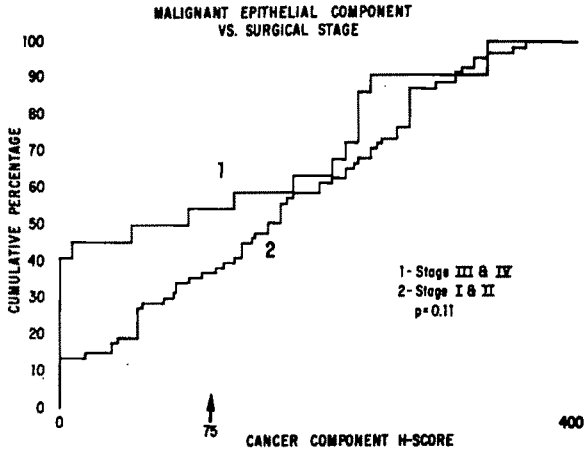


Fig. 6. Cumulative distribution function of malignant component only against surgical stage. *Arrow* denotes threshold of 75. Thirty-seven percent of Stages I and II lesions are estrogen receptor–negative, versus 54% of Stages III and IV.

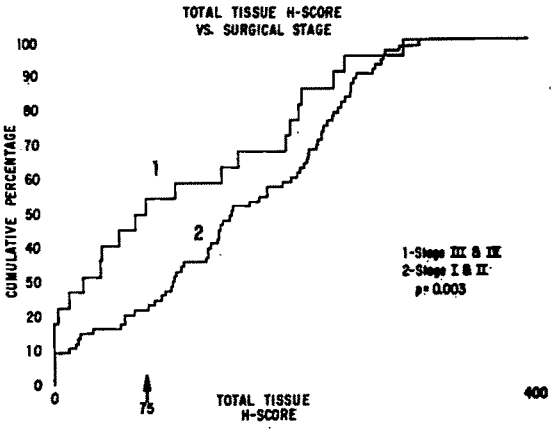


Fig. 7. Cumulative distribution function of total HSCORE versus surgical stage. *Arrow* denotes threshold value of 75. Twenty-two percent of Stages I and II lesions are estrogen receptor–negative, versus 54% of Stages III and IV.

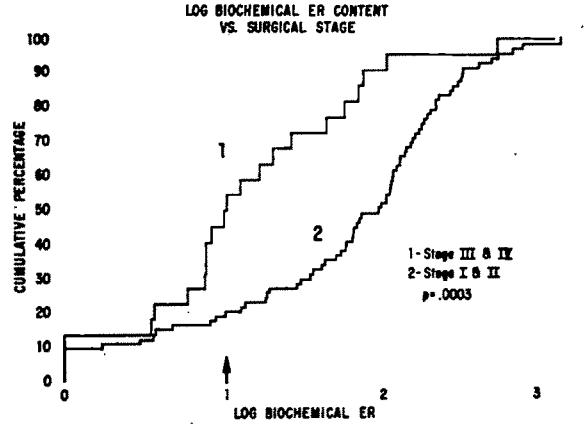


Fig. 8. Cumulative distribution function of log biochemical estrogen receptor *(ER)* against surgical stage. *Arrow* denotes threshold of 10 fmol/mg protein. Twenty percent of Stages I and II lesions are estrogen receptor–negative, versus 45% of Stages III and IV.

and II versus groups of Stages III and IV were significant (p = 0.03, Mann-Whitney; Fig. 7). Distribution of the biochemical estrogen receptor constant was significantly different between stage breakdown (p = 0.003, Mann-Whitney; Fig. 8). There was no difference in the percentages of benign and malignant components present by stage, nor was there any difference in early versus advanced stage cancer.

Comment

In contrast to breast cancer, in which the surrounding mesenchymal elements and benign epithelium contain low or nonmeasurable quantities of sex steroid receptor, the mesenchymal elements in endometrial carcinomas may contain significant quantities. As a consequence, analysis of steroid receptor content of endometrial carcinomas based on biochemical assays of total tissue homogenate may be unreliable for predicting the biologic behavior of the neoplasm. Benign epithelial, stromal, and myometrial elements contain significant amounts of steroid receptor and may contribute quantifiable levels of receptor to the levels measured in individual endometrial carcinoma specimens. Conventional histologic methods can exclude specimens in which significant proportions of the tissue are benign, but cannot provide information about the relative contributions of these various tissue components to the total biochemical estrogen receptor once the tissue is homogenized. For these reasons, a reliable histologic technique for localizing and quantifying sex steroid re-

ceptors is particularly desirable in studying the steroid receptor content of endometrial carcinomas.

The present immunohistochemical technique with H222 Sp γ monoclonal antibody demonstrates good sensitivity and specificity for total HSCORE when compared with standard biochemical estrogen receptor measurements. The total HSCORE discriminated histologic grade with more effective separation of subgroups than was possible with the biochemical estrogen receptor analysis. This separation was even greater when the estrogen receptor contributions of the stromal, myometrial, and benign components were excluded and the malignant epithelial HSCORE was compared with grade (Fig. 3). This cumulative distribution function also demonstrates that grade 2 lesions may be subgrouped into three categories by estrogen receptor content of the malignant component: One subgroup contained little or no estrogen receptor (component HSCORE <75); an intermediate subgroup had a low positive estrogen receptor component HSCORE (75 to 200); and the third subset had a strongly positive component HSCORE (>200). Histologic grading alone could not discriminate any of these subgroups. Therefore, immunohistochemical estrogen receptor evaluation of the cancer component may help determine biologic differentiation and predict tumor behavior better than standard biochemical assays for estrogen receptor that involve whole tissue homogenates, in which discrimination is confounded by the contribution to total estrogen receptor by stroma and myometrium. This concept is underscored when one appreciates that the estrogen receptor content of the myometrium/stroma is often discordant with that of the malignant epithelial component (Fig. 2).

A significant amount of nonepithelial tissue may enter into the biochemical evaluation of estrogen receptor content, thus potentially altering the estimate of biologically important estrogen receptor in the cancerous tissue.[5, 6] In this patient population, both estrogen receptor contribution by percent of HSCORE and percent "benign" contribution by volume of tissue compared with grade and stage were similar between the categories (Fig. 6). Further observations are required to assess the significance of stromal/myometrial estrogen receptor content in predicting the clinical behavior of endometrial carcinoma. We have previously reported a strong prognostic effect of steroid receptor content for predicting disease-free survival in patients with endometrial carcinoma after therapy.[13] Stage correlated well with biochemical estrogen receptor analysis and with the total HSCORE, but did not correlate with HSCORE of the cancer component. Because histologic grade appears to be a biologically relevant factor, and because the component HSCORE of malignant epithelial elements correlated most strongly with grade, one would predict that the cancer component HSCORE would predict stage better than the biochemical estrogen receptor or total HSCORE. In fact, the opposite was observed (Figs. 6 to 8), with biochemical estrogen receptor and total HSCORE relating to stage better than the cancer component HSCORE ($p = 0.003$ and $p = 0.03$ versus 0.11). To evaluate this observation further, the contribution of benign components (stromal, myometrium, and benign epithelium) was analyzed by stage and grade. No differences were noted among groups.

At least two factors may contribute to stage: duration of disease and biologic differentiation of a neoplasm. Histologic differentiation and estrogen receptor content are indirect measures of biologic behavior, but there are likely other aspects not evaluated by these that also contribute to the ability for endometrial carcinoma to metastasize. The duration of disease from its time of inception cannot be determined, but the reverse trend with stage of HSCORE is consistent with the concept that receptor-positive epithelial lesions are slower growing.

A critical observation is the heterogeneity observed between components of endometrial carcinoma. Malignant tissue tends to respond to its environment in an asynchronous fashion (Figs. 1 and 2). In contrast, normal endometrial tissue components tend to respond in synchrony to their hormonal environment.

The immunohistochemical technique described allows the simultaneous determination of the contribution of individual components to the measured estrogen receptor value and the identification of subgroups within populations of lesions and similar histologic differentiation. Data indicate that the estrogen receptor content of these components may be divergent and, further, that the HSCORE of the malignant epithelium does not simply reflect data already provided by stage categorization. Immunohistochemical analysis may therefore represent an important independent variable reflecting tumor differentiation at a biochemical level. Not only does this analysis allow reliable and reproducible quantitation of the estrogen receptor content of the malignant epithelium, it may also allow better evaluation of the interactions of stroma, myometrium, and malignant epithelium, thus permitting the study of malignant tissue response to the hormonal environment in relation to benign tissue response. Finally, this technique allows evaluation of the estrogen receptor content in the cancer component only and therefore may predict biologic behavior better than techniques that involve whole tissue homogenates.

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Colposcopic evaluation of abnormal Papanicolaou smears in pregnancy

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Colposcopy was used to examine 401 pregnant patients with cytologic or clinical abnormalities of the cervix to determine if routine biopsy could be safely omitted except where the colposcopic impression was one of possible invasive carcinoma. A comparison of the antepartum colposcopic impressions with the postpartum histologic diagnosis revealed agreement to within one degree in 87% of patients, with 3% of patients showing a more advanced lesion than that anticipated. Only 2% of patients showed a progression of cytologic abnormalities at postpartum examination; 39% showed marked improvement. Four of nine patients with invasive carcinoma were diagnosed at antepartum colposcopy, with an additional two patients recognized as having invasive cancer at the postpartum colposcopic examination. The omission of routine biopsy is less than ideal if only one antepartum colposcopy is performed, since a considerable period of time may elapse before the patient is seen again postpartum. Those situations in which it may be omitted, together with guidelines for the management of pregnant patients with abnormal cervical ctyologic findings, are presented. (AM J OBSTET GYNECOL 1987;157:932-7.)

Key words: Abnormal Papanicolaou smears, pregnancy, colposcopic evaluation

An abnormal Papanicolaou smear during pregnancy is not uncommon given that the peak incidence[1] for preclinical cervical neoplasia is found in women in their third decade, which is also the time of maximum childbearing activity. Fortunately, few patients who have abnormal Papanicolaou smears during pregnancy are found to have invasive cervical carcinoma. Nevertheless, the occurrence of an abnormal cervical smear during pregnancy produces considerable patient anxiety regarding its significance and the effects that this might have on her pregnancy and fetus.

During the past decade, colposcopy has replaced cervical conization as the method of choice for the evaluation of patients noted to have an abnormal Papanicolaou smear during pregnancy. Although cone biopsy is an excellent method of histologic evaluation of abnormal cervical cytologic findings, the performance of this procedure in pregnancy has produced some difficulties. The physiologic changes that occur with pregnancy, namely, eversion of the squamocolumnar junction, together with increased vascularity and softening of the cervix, often make for greater technical difficulty. In addition, an increased risk of postoperative hemorrhage, premature onset of labor, cervical incompetence, and cervical dystocia are potential complications faced by the patient undergoing cone biopsy while pregnant. These risks can be eliminated by the use of colposcopy. Several publications[2-5] have attested to its safety and reliability. Although the need for colposcopy in the pregnant patient with an abnormal cervical smear is widely accepted, there is a lack of uniformity regarding the investigative protocols that should be used. In particular, more recent reports[2, 5, 6] have suggested that although the risk of significant hemorrhage after colposcopic biopsy is low, biopsies can be omitted if microinvasion or invasive carcinoma is not suspected on the basis of a lesion's colposcopic appearance.

In 1977, we reported[3] our preliminary experience with colposcopy during pregnancy in which all patients had routine biopsy and demonstrated good correlation between the colposcopic impression and the accompanying histologic findings. These results,[3] together with other reports[2, 5-7] that suggested that histologic verification should perhaps be limited to those lesions with features suggestive of invasive carcinoma, led us to modify our investigative protocol along these lines.

This study reports our experience with this approach in which we relied primarily on the colposcopic appearance and avoided routine biopsy in all patients. Biopsy specimens were taken in patients who had lesions that suggested possible invasive carcinoma or in situations in which the appearance was sufficiently nonspecific to make interpretation difficult. Wedge or cone biopsies were subsequently recommended in patients in whom the colposcopic evaluation was equivocal for

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microinvasive disease. This experience was analyzed to see if these concepts could be supported and if further modifications of these guidelines could be made.

Material and methods

Between Jan. 1, 1977 and Dec. 31, 1985, 401 pregnant patients with clinical and/or cytologic abnormalities of the cervix were referred to the Vancouver General Hospital Colposcopy Clinic. At that time, their presenting cytologic findings were reviewed, and colposcopy was performed with the use of routine techniques. Colposcopically directed biopsy specimens were taken only if the lesions seen suggested possible microinvasive or invasive carcinoma. All patients were seen by the authors or fellows under their supervision. Endocervical curettage was not performed, but all patients were assessed regarding whether the transformation zone could be completely outlined, the squamocolumnar junction visualized in its entirety, and all margins of any lesions seen. If these criteria were met and correlation was obtained between the colposcopic and cytologic findings, the examination was classified as satisfactory. If these criteria were not met, the patient was deemed to have an unsatisfactory examination, and further investigation in the form of a wedge or cone biopsy was recommended if the cytologic examination showed suspicious or malignant cells. If wedge biopsy or cervical conization was performed, it was recommended that this be done if possible during the early second trimester.

Patients in whom invasive carcinoma was not apparent by colposcopy were not subjected to further investigation during pregnancy and were advised to return 6 weeks postpartum for repeat cytologic and colposcopic examination. At that time, colposcopically directed biopsy specimens were taken of the most significant area of any lesions seen. The patients referred to the Colposcopy Clinic were seen only for consultation. Further investigation and management of these patients were determined by their physicians.

Patients in whom a diagnosis of occult invasive carcinoma was established during pregnancy were treated by radical hysterectomy and pelvic lymphadenectomy at the earliest opportunity, with consideration given to potential fetal viability in accordance with the wishes of the patient and her spouse. If microinvasive carcinoma was diagnosed or suspected, cone or wedge biopsies were performed, and if the diagnosis was confirmed, a recommendation was made to undergo simple hysterectomy on completion of the pregnancy.

Additional histopathologic findings were available for 106 patients who went on to hysterectomy or cone biopsy after pregnancy, thereby allowing for a comparison of the final pathologic diagnosis with the colposcopic evaluation.

Table I. Age and parity

Age (yr)	*No. of patients*	*Parity*	*No. of patients*
16-20	51	0	207
21-25	116	1	139
26-30	151	2	42
31-35	69	3	8
≥36	14	≥4	5
Total	401		401

Sixty-seven patients had colposcopically directed biopsies while pregnant; two patients also underwent wedge biopsy, and one patient had a cone biopsy performed.

Results

The age and parity of the patients under review are presented in Table I. Three hundred forty-six patients (86%) were para 1 or less, and 318 patients (79%) were 30 years of age or younger, indicating that the population studied consisted essentially of young women who were either nulliparous or of low parity. Indeed, 42% of patients were under 26 years of age, an age when one would not normally expect to see invasive cervical neoplasia. The youngest and oldest patients examined were 16 and 41 years old, respectively.

Table II compares the initial colposcopic impression with the patient's presenting cytologic findings for the 401 patients. Patients with Class I negative cytologic findings were women referred because of a clinically suspicious cervix or persons recently treated for cervical intraepithelial neoplasia whose management was not completed. One hundred eight-six patients had suspicious (Class III) or malignant cells (Class IV) noted on their Papanicolaou smears that were suggestive of carcinoma in situ or early invasive disease. In an additional 105 patients, cells compatible with severe dysplasia or carcinoma in situ (cervical intraepithelial neoplasia III) were reported.

None of the patients who had Class IV cytologic findings had lesions whose features suggested invasive carcinoma. All these patients were thought to have either severe dysplasia or carcinoma in situ.

Sixty-seven patients (17%) had a colposcopically directed biopsy at the time of this initial assessment. In four patients, invasive carcinoma was diagnosed, and 45 of the remaining 63 patients were found to have cervical intraepithelial neoplasia III.

Two hundred twenty-three patients had postpartum colposcopy and biopsy, which allowed for a direct assessment of our ability to predict the underlying histology. These results are noted in Table III. In 87% of patients, the colposcopic impression and the histologic appearance were within one degree of severity. In 24

Table II. Colposcopic impression versus presenting cytology—401 pregnant patients

Antepartum colposcopic impression	*Presenting cytology*					
	Negative Class I	*Mild* Class II*	*Moderate* Class II*	*Marked* Class II*	*Suspicious Class III*	*Malignant Class IV*
Negative	27	4	4	2	1	
Mild dysplasia	3	5	8	5		
Moderate dysplasia	3	7	19	16	10	
Severe dysplasia	2	5	15	57	76	6
Carcinoma in situ	3		5	25	73	16
Microinvasive carcinoma					1	
Occult carcinoma					3	
Total	38	21	51	105	164	22

*Mild, moderate, and marked dyskaryosis.

Table III. Postpartum colposcopic impression versus histologic findings of directed biopsy in 223 patients

Postpartum colposcopic impression	*Histology*					
	Negative	*Mild dysplasia*	*Moderate dysplasia*	*Severe dysplasia*	*Carcinoma in situ*	*Occult carcinoma*
Negative	9	1				
Mild dysplasia	4	6	1		1	
Moderate dysplasia	2	6	7	7	4	
Severe dysplasia	5	12	14	32	43	
Carcinoma in situ	2	1	2	12	50	
Occult carcinoma					1	1
Total	22	26	24	51	99	1

Table IV. Colposcopic evaluation versus final histologic diagnosis in 106 patients

Colposcopic evaluation	*Final histologic diagnosis*						
	Negative	*Mild dysplasia*	*Moderate dysplasia*	*Severe dysplasia*	*Carcinoma in situ*	*Microinvasive carcinoma*	*Occult carcinoma*
Moderate dysplasia			1				
Severe dysplasia	1	1	4	9	5		
Carcinoma in situ	1	1		5	69		3
Microinvasive carcinoma						1	
Occult carcinoma							5
Total	2	2	5	14	74	1	8

patients (11%), the histologic diagnosis was less severe than that anticipated by the colposcopic impression, and in five patients (2%), a more advanced lesion was found on biopsy examination than what was predicted.

A similar analysis was done after review of the histologic findings of the 106 patients who had additional tissue specimens for evaluation either from conization or hysterectomy. This comparison is presented in Table IV and shows that once again the predictive ability was similar to that noted in Table III and the tendency of colposcopy to overcall the lesser forms of cervical intraepithelial neoplasia. The only notable difference is with the number diagnosed with invasive cancer. Five of the nine patients with invasive cancer shown in Table IV were diagnosed at subsequent postpartum investigation. Two patients had colposcopic features that suggested invasive carcinoma, and this was confirmed by directed biopsy in one patient. In the other patient, the postpartum biopsy examination did show carcinoma in situ, which was similar to the antepartum diagnosis, but now the lesion was not visualized completely, and its appearance was suggestive of invasive carcinoma; thus a diagnostic cone was recommended in cases in which an occult cancer was detected. Two patients had postpartum conizations, one without further colposcopic assessment and in another instance a cesarean-hysterectomy was performed because of a placenta accreta. All three of these women had occult in-

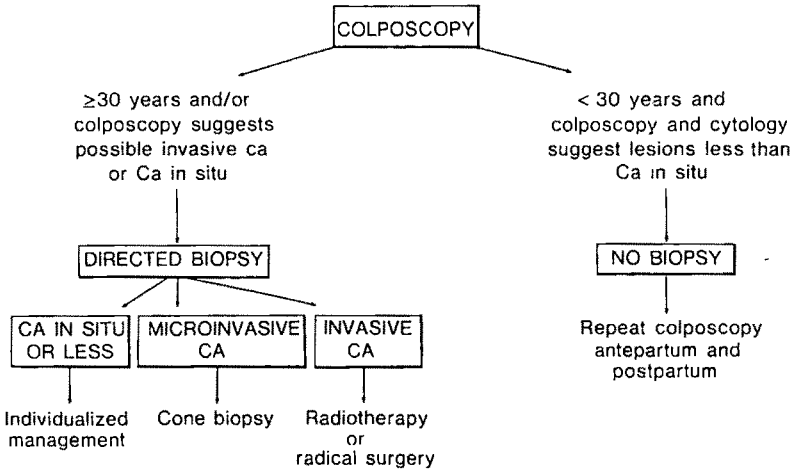


Fig. 1. Guidelines for management of patients with abnormal smears in pregnancy. (*CA* = Cancer.)

Table V. Antepartum versus postpartum cytology

Antepartum cytology	*Total No. of patients*	*Postpartum cytology*					
		Negative Class I	*Dyskaryosis Class II*			*Suspicious Class III*	*Malignant Class IV*
			Mild	*Moderate*	*Marked*		
Class I negative	31	28	1			2	
Class II							
Mild dyskaryosis	17	8	4	1	3	1	
Moderate dyskaryosis	43	20	6	8	6	3	
Marked dyskaryosis	93	27	13	16	19	16	2
Class III suspicious	145	41	16	9	23	53	3
Class IV malignant	19	5	1	1	1	7	4
Total	348	129	41	35	52	82	9

vasive carcinomas and represent patients in whom the diagnosis of malignancy was not made or anticipated colposcopically.

In reviewing the patients' presenting cytologic findings with histologic specimens, which were obtained either colposcopically or at the time of conization or hysterectomy, it was noted that 73% of patients had cytologic findings predictive of the underlying disease. In 18% of patients, the histologic findings were less advanced than had been anticipated, with the remaining 9% of patients showing a histologically more advanced lesion than had been predicted by the cervical cytology report.

Table V compares the results of the antepartum and postpartum cytologic examination in 348 patients in whom this information was available. In 203 patients (58%), the antepartum and postpartum cytologic findings were similar; however, 134 patients (39%) exhibited a marked improvement in the degree of cytologic abnormality during the postpartum period. In contrast, only 11 patients (2%) had deterioration in their cytologic atypicality during pregnancy.

Table VI. Current status of 401 patients

Status	*No. of patients*
Postpartum colposcopy and biopsy	223
Cytologic follow-up only	98
Cone biopsy or hysterectomy	30
Cryotherapy	5
Undelivered	32
Lost to follow-up	13
Total	401

The current status of the 401 patients under review for this study is presented in Table VI. In 30 patients, the physicians elected to proceed directly to conization or hysterectomy postpartum without further cytologic or colposcopic investigation. In addition, 76 patients also had a hysterectomy or conization after postpartum

Table VII. Patients with invasive carcinoma

No.	*Patient*	*Age (yr)*	*Gestational age (wk)*	*Presenting cytology (Class)*	*Colposcopic impression antepartum*	*Colposcopic impression postpartum*	*Final histology*
1	C. V.	37	23	III Suspicious	Occult	–	Occult
2	C. J.	26	37	III Suspicious	Occult	–	Occult
3	W. T.	26	10	III Suspicious gland cells	Occult	–	Occult adenocarcinoma
4	P. L.	37	20	III Suspicious	Microinvasive	–	Microinvasive
5	V. P. H.	40	10	II Marked dyskaryosis	CIS	Occult	Occult
6	F. D.	27	15	III Suspicious	CIS	? Occult unsatisfactory examination	Occult
7	T. H.	27	14	II Suspicious	CIS	–	Occult
8	S. G.	37	28	II Marked dyskaryosis	CIS	–	Occult
9	C. S.	32	14	III Suspicious	CIS	CIS	Occult

RHND = Radical hysterectomy and node dissection; CIS = carcinoma in situ; TAH = total abdominal hysterectomy; bx = biopsy; PP = postpartum.

colposcopy and biopsy. Thirty-two patients were undelivered or less than 6 weeks' postpartum at the time of this evaluation, and in 13 patients, no further follow-up records could be obtained. Further management of the remaining 352 patients (88%) is also listed in Table VI.

A summary of the clinical findings in those patients with invasive carcinoma in this series is shown in Table VII. Five of the nine patients were older than age of 30 years, with a mean age of 32 years. Only 83 patients were in this age group. These five patients represent 6% of the 83 persons over 30 years old. In contrast, only four (1.3%) of 318 patients who were 30 years of age or less had invasive carcinoma. Furthermore, 58, or 70% of the 83 patients over the age of 30 years, had suspicious Class III or malignant Class IV cytologic findings on presentation. Of the 318 patients under the age of 30 years, 110, or 35%, had suspicious or malignant cytologic findings. Not only were patients 30 years of age or older twice as likely to have Class III or IV smears, but they were also approximately five times more likely to have invasive carcinoma.

All patients who underwent colposcopically directed biopsies during pregnancy did so without hemorrhagic complications. Premature labor or pregnancy loss did not occur in any patient.

Comment

The results of this study confirm that invasive cervical carcinoma is uncommon in pregnancy,[8] particularly in a heavily screened population such as ours. Nonetheless, whether such a lesion is present is the critical question that one must ask when confronted with a pregnant patient with abnormal cervical cytologic findings. Delay in the diagnosis of an early invasive carcinoma may result in a potentially curable lesion that progresses over the course of the pregnancy to the point where a successful outcome is no longer possible.

Analysis of the study data revealed that a proportionately larger number of patients over the age of 30 years had invasive cancer than those younger. Similarly, this older age group was also much more likely to have suspicious or malignant-appearing cells on their cervical smears than were younger patients. Thus the combination of age 30 years or more with a Class III or IV Papanicolaou smear should alert the physician that one is dealing with a particularly high-risk person who should probably have routine biopsy, even if the colposcopic appearance suggests only carcinoma in situ or a lesser lesion. The results of this and previous studies have shown that the risk of hemorrhage from a colposcopically directed biopsy is extremely low.

Routine biopsy need not be performed in all patients but to rely solely on the colposcopic appearance of the lesion presupposes that a lesion will have sufficient characteristics to permit its identification as such and that the colposcopist possesses the requisite expertise to allow for this recognition. In the current series, five patients with invasive carcinoma were not diagnosed until the postpartum period. In two patients, the colposcopic appearance postpartum was such as to suggest the diagnosis. Two patients did not have postpartum colposcopy, and in one patient the postpartum colposcopic impression and biopsy once again showed carcinoma in situ. This patient went on to have a simple abdominal hysterectomy at which time the diagnosis was made. It is possible that some of these lesions may have progressed from an intraepithelial to an invasive carcinoma over the course of the pregnancy, particularly since all were small-volume tumors. It is also possible, however, that in some of these patients an early invasive carcinoma was present at the outset and went

Comment
RHND at 38 wk
RHND at 38 wk
RHND at 12 wk, adenocarcinoma
Wedge bx = microinvasive, PPTAH = CIS
PP Pap Class IV; PP colpo bx = invasive, RHND
PP colposcopic bx = CIS, PP cone = occult, RHND
Placenta accreta–cesarean hysterectomy = occult
No postpartum colposcopy, PP cone = occult
PP colposcopic bx = CIS, TAH = occult

unrecognized at the time of the antepartum colposcopic assessment. Had routine biopsy been done, it may have had the advantage of alerting the physician to the need for further tissue investigation and on occasion may have shown an unexpected invasive carcinoma. All the patients who were diagnosed in the postpartum period had only one antepartum colposcopic examination early in the pregnancy. If further colposcopic examinations in these patients had been performed during the course of the pregnancy and if changes in the lesions were present, they may have been noted and the diagnosis made earlier.

In general, if an invasive carcinoma is present but not diagnosed at colposcopic examination, this is usually attributed to inexperience or colposcopist error. Sugimori et al.,[9] reporting on the colposcopic findings in microinvasive carcinoma of the cervix, reported that 7% of such patients had no colposcopic abnormalities suggesting neoplasia. Other reports[10-12] have also suggested that some lesions may not have features sufficiently atypical to permit easy recognition as early invasive carcinomas. Physicians are reluctant to report cases in which invasive carcinomas were not recognized at colposcopy for fear that the problem of nonrecognition would be attributed to the physician whereas it may be the technique that is at fault. If persons performing colposcopy adhere to established investigative protocols, colposcopist-related errors will be minimal.

Our experience to date with patients who have abnormal cervical cytologic findings during pregnancy leads us to believe that these are a special category of persons requiring specific management guidelines. This experience has led us to adopt the following guidelines: (1) All lesions with features suggestive of invasive carcinoma or carcinoma in situ should undergo biopsy examination at the initial visit, and if lesser pathologic findings are noted, repeat colposcopy and biopsy are performed in 4 weeks. (2) All patients 30 years of age or older with Class III or IV cervical cytologic findings are to have directed biopsies, even if the lesion seen is suggestive of a lesser disease than carcinoma in situ. (3) Patients should be seen as early as possible during pregnancy and then for 2 to 3 additional visits over the course of the pregnancy, depending on the gestational age of the patient. (4) Patients with suspicious or malignant cytologic findings (Class III or IV) who have an unsatisfactory colposcopic examination should be examined under anesthesia with colposcopy, and at that time a decision should be made regarding the need for wedge biopsy or cervical conization. (5) All patients should be seen 6 to 8 weeks' postpartum for repeat cytology, colposcopy, and biopsy. These guidelines are summarized in Fig. 1.

Although the vast majority of these patients will not have cancer, particularly the young patient with mild cytologic and colposcopic abnormalities and thus will not benefit from routine biopsy, its omission is less than ideal, since there is little doubt that colposcopically directed biopsy during pregnancy can be performed easily with minimal risk and complications.

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Acute effects of exercise on plasma catecholamines in sedentary and athletic women with normal and abnormal menses

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Norepinephrine plays a role in the regulation of luteinizing hormone secretion and may therefore be involved in the etiology of exercise-induced menstrual dysfunction. This study evaluated both intraexercise and postexercise responses of epinephrine, norepinephrine, and dopamine in sedentary women and women runners with normal and abnormal menstruation. Five eumenorrheic nonrunners and five eumenorrheic, four oligomenorrheic, and five amenorrheic runners were evaluated on 2 consecutive days. On day 1, the women cycled on a bicycle ergometer against an increasing work load until exhaustion, and on day 2, the women underwent a submaximal exercise regimen. Serial blood draws were taken at specified time intervals during intraexercise and postexercise periods on both days. The data collected during exercise for all groups showed that epinephrine and norepinephrine had a sixfold to sevenfold rise on day 1 and had a threefold rise on day 2. Dopamine increased twofold during both exercise protocols. On day 1 norepinephrine displayed a significantly higher percentage change from baseline to peak levels for oligomenorrheic and amenorrheic runners than for eumenorrheic runners and sedentary women. This latter finding is consistent with the hypothesis that periodic marked elevations in norepinephrine levels during maximal exercise may interfere with pulsatile luteinizing hormone release and hence may play a role in the occurrence of menstrual dysfunction in women runners. (Am J Obstet Gynecol 1987;157:938-44.)

Key words: Catecholamines, luteinizing hormone, women runners

Menstrual dysfunction has been found to occur in some female runners after the onset of training. A variety of hormonal alterations have been implicated as contributing to the dysfunction. Gonadotropin,[1] prolactin,[2] endorphin,[3] and catecholamine[4] levels have been found altered after the onset of regular or vigorous exercise. Studies in women athletes have shown cathecholamine rises after exercise in relation to increasing activity levels similar to men, but none have formally addressed the association between elevated catecholamine levels and exercise-related menstrual disturbance.

During strenuous exercise norepinephrine and epinephrine may attain levels that are similar to those obtained during norepinephrine and epinephrine infusions, which can produce hemodynamic and metabolic changes.[5] Recent works have implicated norepinephrine[6] in altering the pulsatility of luteinizing hormone release, which is critical for normal cyclic ovulatory function. This study examines the influences of maximal and submaximal exercise regimens on catecholamine secretion in sedentary women and women runners with and without menstrual dysfunction.

Material and methods

Subjects. Nineteen women (mean age 25.4 years, range 19 to 35 years) who gave informed consent were admitted to the Clinical Research Center at the Ohio State University Hospitals on two consecutive afternoons. This group of women had previously been involved with a study evaluating growth hormone and prolactin levels.[2] They were initially screened by a detailed questionnaire and underwent a complete physical examination. Nonrunning women (n = 5) had normal menstrual histories and did not exercise excessively. No prescribed medications or oral contraceptives were used by any of the subjects for at least 6 months before the study. Runners were required to have run a minimum of 25 miles/wk for at least 12 months before the study. Four of the five eumenorrheic runners had competed in marathons and 10 km races on a regular basis,

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Table I. Subject profile*

	Nonrunning women (n = 5)	*Eumenorrheic running women (n = 5)*	*Oligomenorrheic running women (n = 4)*	*Amenorrheic running women (n = 5)*
Age (yr)	26.8 ± 5.2	28.4 ± 5.7	21.0 ± 4.2	26.0 ± 5.0
Miles/wk		35.0 ± 16	32.0 ± 5.4	36.0 ± 13
Age at menarche (yr)	11.8 ± 6.6	13.4 ± 1.5	13.5 ± 0.6	12.3 ± 0.4
Wt (kg)	54.8 ± 6.6	53.4 ± 6.1	58.6 ± 4.0	46.3 ± 2.6†
Ht (inches)	65 ± 1.9	63.4 ± 6.1	67.3 ± 2.6	63 ± 2.4
% fat	22.5 ± 5.0	20.6 ± 1.7	18.2 ± 5.4	17.5 ± 5.5

*Values are the mean ± SD.

†$p < 0.05$ compared with the other three groups.

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Table II. Baseline endocrine profile*

	Nonrunning women (n = 5)	*Eumenorrheic running women (n = 5)*	*Oligomenorrheic running women (n = 4)*	*Amenorrheic running women (n = 5)*
Prolactin (ng/ml)	6.0 ± 2.8	6.8 ± 4.1	4.9 ± 2.4	5.4 ± 3.6
Follicle-stimulating hormone (mIU/ml)	8.0 ± 4.4	9.0 ± 1.9	7.6 ± 1.6	7.5 ± 2.4
Luteinizing hormone (mIU/ml)	2.6 ± 1.1	2.3 ± 1.3	2.9 ± 0.8	2.2 ± 1.4
Estradiol (pg/ml)	54.0 ± 20	56.0 ± 35.2	40.3 ± 9.6	22.6 ± 11.2
Progesterone (ng/ml)	0.57 ± 0.2	0.58 ± 0.8	0.60 ± 0.8	0.52 ± 1.3

*Values are the mean ± SD.

Modified from Chang FE, Dodds WG, Sullivan M, Kim MH, Malarkey WB. The acute effects of exercise on prolactin and growth hormone secretion: comparison between sedentary women and women runners with normal and abnormal menstrual cycles. J Clin Endocrinol Metab 1986;62:551-6. Reprinted with permission.

whereas three of the four oligomenorrheic runners and four of the five amenorrheic runners also regularly competed in similar distance races. Eumenorrheic runners had regular cycles (mean 28.4 days, range 28 to 29 days), oligomenorrheic runners had cycle intervals of 55.5 days (range 45 to 72 days), and amenorrheic runners did not menstruate for an average of 17 months (range 6 to 36 months). All runners had regular menstrual cycles before the start of serious training. The subjects were similar in age, height, percentage body fat, age of menarche, and weekly running mileage. However, the amenorrheic runners were lower in weight compared with the others ($p < 0.05$, Table I). The runners with menstrual dysfunction had previous hormonal evaluations that ruled out other causes for their irregular menses; they also had no associated medical conditions that could be responsible for their menstrual dysfunction.

Study protocol. All subjects with the exception of the amenorrheic runners were admitted during the midfollicular phases (days 5 to 10) of their menstrual cycles. The subjects were asked to avoid caffeine on the days of the study. They had low-protein breakfasts and did not eat or drink for 4 hours before the study. Each subject had skin fold thicknesses measured from the triceps, suprailiac, and anterior midthigh regions using a Lange caliper. Percentage of body fat was then determined by the method of Pollack et al.[7] All exercise studies were initiated between the hours of noon and 3:00 PM. An 18-gauge heparin lock was placed in the forearm 45 minutes before the study. The subjects were asked to stand for 10 minutes before measurements of baseline blood pressure and heart rate were recorded. Exercise was performed on a Quinton Model 844 Unitwork upright bicycle ergometer. Subjects sat on the bicycle for 30 minutes before the start of the exercise. On day 1, the subjects were asked to cycle starting at a work load of 200 kg-m/min, which was increased by 200 kg-m/min every 2 minutes until the subject could not continue because of exhaustion. Heart rate was monitored by a heart monitor (Electronics for Medicine, White Plains, N.Y.) continually throughout the exercise and rest phases. Blood pressure was taken with a blood pressure cuff and recorded every 2 minutes. Blood was obtained at baseline and every 8 minutes during the exercise period; a final sample was obtained at the time of exhaustion. Blood was obtained at 2, 5, 15, 30, and 60 minutes during the postexercise period as the subject sat quietly in an upright position.

The second day of the study was designed to assess

Table III. Epinephrine (pg/ml) response to maximal exercise*

	Nonrunning women	*Eumenorrheic running women*	*Oligomenorrheic running women*	*Amenorrheic running women*
Exercise (min)				
0	55 ± 9	62 ± 12	71 ± 17	54 ± 8
8	128 ± 33	93 ± 16	91 ± 19	83 ± 13
Max	454 ± 84	490 ± 174	490 ± 217	323 ± 83
Postexercise (min)				
2	141 ± 21	190 ± 18	213 ± 64	125 ± 22
5	82 ± 11	101 ± 32	150 ± 56	88 ± 14
15	63 ± 8	78 ± 17	102 ± 9	73 ± 15
30	69 ± 7	92 ± 27	97 ± 18	57 ± 5
60	59 ± 13	75 ± 18	81 ± 8	56 ± 3

*Values are the mean ± SEM.

Table IV. Epinephrine (pg/ml) response to submaximal exercise*

	Nonrunning women	*Eumenorrheic running women*	*Oligomenorrheic running women*	*Amenorrheic running women*
Exercise (min)				
0	69 ± 16	62 ± 14	77 ± 8	56 ± 11
20	112 ± 22	114 ± 28	107 ± 18	97 ± 19
45	226 ± 45	199 ± 44	157 ± 25	174 ± 24
Postexercise (min)				
2	131 ± 25	111 ± 15	113 ± 20	108 ± 23
5	81 ± 13	66 ± 10	91 ± 17	71 ± 9
15	83 ± 13	71 ± 11	66 ± 6	69 ± 14
30	68 ± 19	55 ± 9	54 ± 6	64 ± 7

*Values are the mean ± SEM.

responses to a submaximal aerobic exercise regimen. The method of Karvonen et al.[8] was used to approximate percentage Vo_2 max and this method is accurate to within ±12%. Sixty percent of the difference between the baseline heart rate and maximal heart rate achieved on day 1 was added to the baseline heart rate to calculate the heart rate to be attained. The subject cycled for 30 minutes against variable resistance to maintain this calculated heart rate at approximately 60% Vo_2 max. She then cycled at a heart rate approximating 80% Vo_2 max for another 15 minutes. Blood was obtained via the heparin lock 0, 20, and 45 minutes during the exercise phases. At the end of the 45-minute exercise session, the subject sat in an upright position for 30 minutes while blood sampling was performed at 2, 5, 15, 30 minutes of the postexercise period.

Assay. The catecholamine levels were analyzed by high-performance liquid chromatography with electrochemical detection (Bioanalytical Systems, Inc., West Lafayette, Indiana). Samples were extracted with aluminum before assay. Recovery of the internal standard, 3,4-dihydroxybenzylamine, was 59% ± 8% (mean ± SD) during the assay period. Levels of prolactin, estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone were measured by radioimmunoassay. The intra-assay and interassay coefficients of variation were prolactin 7.0%, 12.0%; estradiol 8.6%, 13.4%; progesterone 9.0%, 13.6%; luteinizing hormone 8.6%, 13.4%; and follicle-stimulating hormone 9.5%, 15.5%, respectively. Plasma lactate was measured by an autoanalyzer.

Statistical methods. All data were entered into the data management CLINFO system of the Clinical Research Center. One-way analysis of variance with the Newman-Keuls aftertest was performed for patient profile and cardiovascular data. Either one-way analysis of variance or nonpaired Student *t* tests were performed on nontransformed and log-transformed data obtained during the exercise and rest periods. The results were considered significant at $p \leq 0.05$.

Results

Baseline endocrine profile. The baseline endocrine profile was similar for the four groups (Table II). There were no significant differences in baseline prolactin, estradiol, follicle-stimulating hormone, and luteinizing hormone values or in the luteinizing hormone to follicle-stimulating hormone ratios, although the estradiol levels were lower in the amenorrheic women. All the cycling women were evaluated during the follicular phase as confirmed by progesterone levels <1.0 ng/ml.

Cardiovascular. Baseline heart rates of nonrunning

Table V. Norepinephrine (pg/ml) response to maximal exercise*

	Nonrunning women	*Eumenorrheic running women*	*Oligomenorrheic running women*	*Amenorrheic running women*
Exercise (min)				
0	457 ± 49	416 ± 57	392 ± 70	398 ± 93
8	830 ± 185	671 ± 102	505 ± 43	619 ± 72
Max	2047 ± 375	2324 ± 437	2959 ± 469	3185 ± 820
Postexercise (min)				
2	1025 ± 156	1491 ± 186	1527 ± 426	1074 ± 257
5	782 ± 139	780 ± 130	1049 ± 280	661 ± 167
15	456 ± 89	534 ± 79	697 ± 265	414 ± 54
30	393 ± 81	477 ± 77	570 ± 199	326 ± 41
60	351 ± 51	584 ± 51	398 ± 85	374 ± 76

*Values are the mean ± SEM.

Table VI. Norepinephrine (pg/ml) response to submaximal exercise*

	Nonrunning women	*Eumenorrheic running women*	*Oligomenorrheic running women*	*Amenorrheic running women*
Exercise (min)				
0	425 ± 61	466 ± 59	372 ± 51	390 ± 60
20	703 ± 108	809 ± 59	729 ± 67	731 ± 57
45	1238 ± 142	1369 ± 202	1143 ± 164	1553 ± 552
Postexercise (min)				
2	901 ± 163	892 ± 84	906 ± 68	962 ± 241
5	608 ± 67	674 ± 65	706 ± 85	507 ± 80
15	461 ± 35	490 ± 51	383 ± 50	382 ± 30
30	433 ± 59	413 ± 65	354 ± 64	375 ± 50

*Values are the mean ± SEM.

women, 74.0 ± 2.2 (±SEM) bpm, were significantly higher ($p < 0.05$) than the baseline heart rates of the eumenorrheic running women (59.0 ± 5.3 bpm at 6:00 PM), the oligomenorrheic running women (59.3 ± 3.4 bpm at 6:00 PM), and the amenorrheic women (57.5 ± 2.9 bpm at 6:00 PM). All achieved a maximum heart rate higher than 160 bpm on the day of maximal exercise, and there was no difference in the maximum heart rate achieved. The time required to achieve exhaustion on day 1 varied among the four groups of women (nonrunning eumenorrheic women, 12 to 16 minutes; eumenorrheic women, 16 to 25 minutes; oligomenorrheic women, 20 to 24 minutes; and amenorrheic women, 16 to 20 minutes).

Plasma lactate was measured before exercise and at 5 minutes of the postexercise period. Plasma lactate levels increased sixfold to ninefold in all subjects exercising on day 1, reflecting significant anaerobic work. Mean ± SEM lactate levels after exercise ranged from 5.9 ± 1.4 to 8.8 ± 1.2 mEq/L in the four groups and were significantly higher than baseline levels ($p < 0.05$) for all groups on day 1. The plasma lactate levels after submaximal exercise were minimally increased in each group (mean values ranging from 1.5 ± 0.4 to 2.7 ± 0.5 mEq/L), which confirmed that primarily aerobic work was performed. Notably, only the eumenorrheic running women had significantly higher lactate levels after submaximal exercise compared with baseline level (2.3 ± 0.4 versus 1.0 ± 0.1 mEq/L; $p < 0.05$).

Plasma epinephrine

Day 1. Plasma epinephrine levels increased during maximal exertion sixfold to eightfold above resting levels in all four study groups ($p < 0.001$; Table III) and returned to baseline by 60 minutes. The peak elevation of epinephrine in response to maximal exertion was similar for all groups.

Day 2. Plasma epinephrine levels for all groups increased twofold to threefold from baseline levels for submaximal exercise ($p < 0.001$; Table IV). The epinephrine responses were less than half of those found during maximal exertion. Epinephrine levels returned to baseline by 15 minutes, and no differences were found in epinephrine responses between groups.

Plasma norepinephrine

Day 1. Plasma norepinephrine values increased fivefold to eightfold for the four groups after maximal exertion ($p < 0.001$; Table V). Women runners with menstrual dysfunction had a significantly higher percentage change from baseline to peak levels for norepinephrine levels than did eumenorrheic runners and control subjects ($p < 0.02$, Fig. 1). Thirty minutes after

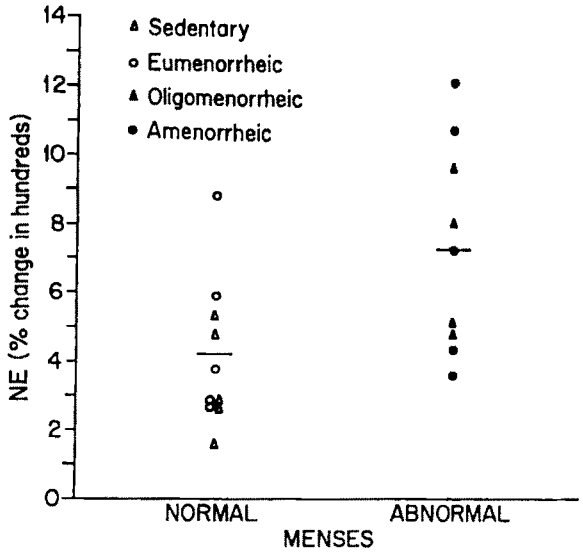


Fig. 1. Women runners with abnormal menses had significantly ($p < 0.02$) elevated plasma levels of norepinephrine *(NE)* during maximal exercise when compared with sedentary women and women runners with normal menses. The data are expressed as percentage increase of norepinephrine over preexercise levels.

exercise, norepinephrine levels had returned to basal concentrations for all groups.

Day 2. Plasma norepinephrine concentrations were threefold greater than their respective baseline values at the end of the submaximal exertion periods for all groups ($p < 0.001$; Table VI). The mean norepinephrine levels returned to basal concentrations 30 minutes after exercise in each group. No differences in norepinephrine levels were found between the groups.

Plasma dopamine

Days 1 and 2. Plasma dopamine levels increased twofold for both exercise protocols ($p < 0.001$, Tables VII and VIII), with recovery to baseline occurring within 15 minutes for both days. There were no significant differences in peak dopamine levels between groups.

Comment

The women in this study had dramatic increases in their plasma norepinephrine and epinephrine levels during maximal exercise. Several women runners achieved norepinephrine and epinephrine values as high as 4000 to 6100 and 1000 to 1100 pg/ml, respectively, a ninefold increase for norepinephrine and a 15-fold increase for epinephrine over basal levels. These levels are higher than previously reported,[4] most likely because the samples were obtained during exercise rather than immediately after its cessation. In our study plasma norepinephrine and epinephrine levels decreased by more than 50% at 2 minutes after exercise. Catecholamine levels were significantly higher for the exercise-to-exhaustion regimen than for the submaximal exercise regimen ($p < 0.001$). This finding is consistent with a report showing that anaerobic activities place a greater demand on the sympathoadrenal system than do aerobic activities.[9]

During the maximal but not submaximal exercise regimen, the percentage change of norepinephrine from baseline to peak levels was greater for oligomenorrheic and amenorrheic runners than for eumenorrheic runners and sedentary women ($p < 0.02$). Graded infusion studies in humans have demonstrated that norepinephrine in the 1500 to 2000 pg/ml range can produce alterations in the metabolic and cardiovascular systems.[5] During vigorous exercise norepinephrine assumes the role of a hormone reaching levels capable of impairing carbohydrate and lipid metabolism, in addition to increasing heart rate and cardiovascular tone.

Norepinephrine has been found to play a dual role in the control of luteinizing hormone release, since both stimulation[10] and inhibition[11, 12] of luteinizing hormone secretion have been described in animal studies. The ability of norepinephrine to have both excitatory and inhibitory effects on luteinizing hormone release has been attributed to its effect on different subpopulations of adrenoceptors, α_1,[13] α_2,[11] and β.[14] A certain basal level of adrenergic neurotransmission is thought to be required to activate the excitatory α-adrenergic receptor population on the luteinizing hormone–releasing hormone neuron for the maintenance of normal pulsatile luteinizing hormone release.[13] However, a relatively large increase in norepinephrine, such as intraventricular infusion[11] or electrical stimulation of brain norepinephrine pathways,[12] may result in an abrupt interruption of the normal pulsatile luteinizing hormone release. Perhaps the women runners in this study with menstrual dysfunction undergo a similar disruption in luteinizing hormone pulsatility from an acute rise in norepinephrine during maximal exercise. The data of Veldhuis et al.[15] support this hypothesis in that their women runners with exercise-associated menstrual dysfunction were found to have a decrease in luteinizing hormone pulse frequency compared with eumenorrheic runners.

An additional explanation is available regarding how an increase in plasma catecholamines could alter the menstrual function of these women runners. Catecholamines have been reported to inhibit the degradation of β-endorphins by blocking the activity of enkephalin aminopeptidase. This finding is potentially important in that β-endorphins can reduce the pulse frequency of luteinizing hormone release[16] and therefore provide an additional mechanism by which elevated plasma catecholamine levels during exercise could influence menstrual function.

Table VII. Dopamine (pg/ml) response to maximal exercise*

	Nonrunning women	*Eumenorrheic running women*	*Oligomenorrheic running women*	*Amenorrheic running women*
Exercise (min)				
0	69 ± 12	95 ± 11	92 ± 20	81 ± 14
8	76 ± 12	110 ± 9	81 ± 18	108 ± 18
Max	135 ± 41	106 ± 17	209 ± 53	166 ± 50
Postexercise (min)				
2	127 ± 40	141 ± 16	178 ± 42	95 ± 26
5	81 ± 15	99 ± 22	180 ± 44	74 ± 15
15	87 ± 13	86 ± 15	121 ± 13	62 ± 9
30	70 ± 9	104 ± 17	132 ± 29	76 ± 14
60	70 ± 10	101 ± 24	93 ± 21	76 ± 14

*Values are the mean ± SEM.

Table VIII. Dopamine (pg/ml) response to submaximal exercise*

	Nonrunning women	*Eumenorrheic running women*	*Oligomenorrheic running women*	*Amenorrheic running women*
Exercise (min)				
0	60 ± 6	81 ± 16	84 ± 12	63 ± 11
20	93 ± 24	110 ± 15	107 ± 24	128 ± 12
45	123 ± 30	111 ± 17	134 ± 29	136 ± 11
Postexercise (min)				
2	84 ± 18	129 ± 29	119 ± 32	114 ± 27
5	67 ± 5	89 ± 15	98 ± 17	78 ± 13
15	62 ± 6	77 ± 6	95 ± 27	87 ± 17
30	67 ± 16	67 ± 7	93 ± 21	87 ± 14

*Values are the mean ± SEM.

In summary, we have found that women runners have a marked elevation of plasma catecholamine levels during maximal exercise and that norepinephrine levels were significantly higher in the runners with menstrual dysfunction. We propose that norepinephrine may work in concert with other factor(s) to produce menstrual dysfunction in women runners.

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Treatment of menorrhagia with meclofenamate sodium

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A double-blind, placebo-controlled crossover trial was undertaken to determine the efficacy of meclofenamate sodium in the treatment of menorrhagia. Twenty-nine patients who had a baseline menstrual blood loss greater than 60 ml received 2 months' each of meclofenamate sodium, 100 mg by mouth, three times a day, or a placebo. The mean menstrual blood loss was reduced from 141.6 ± 15.9 ml at baseline to 69.0 ± 6.3 ml during treatment cycles but remained increased during placebo cycles (135.6 ± 11.3 ml). The symptoms of dysmenorrhea, backache, and headache were significantly reduced only during active drug periods. The number of days of flow and pads or tampons used was also reduced during drug cycles but not during placebo cycles. Overall, 26 of the 29 patients evaluated had a reduction in menstrual blood loss with the use of meclofenamate sodium. It appears that many women with unexplained menorrhagia may benefit from this treatment. (Am J Obstet Gynecol 1987;157:944-50.)

Key words: Menorrhagia, prostaglandin inhibitors, meclofenamate sodium

The average blood loss at the time of menses is estimated to be between 20 and 60 ml. There appears to be an increased incidence of iron-deficiency anemia if blood loss exceeds this amount.[1] A statistically significant number of women will have hemoglobin concentrations of <12 gm/dl when menstrual blood loss exceeds 80 ml.[1] Many of these women have no identifiable, organic, or endocrinologic explanation for their menorrhagia.

The endometrium of women with menorrhagia has been found to have higher levels of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ when compared with women with normal menses.[2] Furthermore, it has been hypothesized that the increase in prostaglandin E_2 production and binding coupled with the vasodilatory action of prostaglandin E_2 may be the pathophysiologic explanation for the menorrhagia in patients with an otherwise unknown cause for the blood loss.[3]

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The subgroup of prostaglandin inhibitors known as the fenamates act by both inhibiting the biosynthesis of the cyclic endoperoxides and by blocking prostaglandin E_2 and prostaglandin $F_{2\alpha}$ directly at the receptor sites. Anderson evaluated the effect of mefenamic acid therapy in 22 patients with menstrual blood loss of >80 ml. Twenty patients demonstrated a 2% to 78% reduction in blood loss with therapy.[4] Fraser et al.[5] undertook a 4-cycle, double-blind, randomized, placebo-controlled crossover trial and found that in the 30 patients studied, the mean blood loss that occurred during mefenamic acid treatment cycles was 28.1%.

With these findings in mind, we sought to determine the efficacy and safety of meclofenamate sodium (Meclomen). This study was undertaken to determine whether meclofenamate sodium was effective in treating women with menorrhagia of unknown etiology with the use of 4-month double-blind crossover trial.

Patient selection

Patients who were 16 to 42 years old with a definite history of menorrhagia and a negative pregnancy test were entered into a 2-month observation period to confirm the diagnosis and evaluate the possible cause. During this period, the patients underwent a complete history and physical and a late luteal phase endometrial biopsy. In addition, a complete blood count, erythrocyte sedimentation rate, serum ferritin, serum

glutamic-pyruvic transaminase, blood urea nitrogen, T_3, T_4, and urinalysis were performed. They collected all sanitary napkins and tampons during two menstrual periods, which were subsequently assayed for menstrual blood loss by the alkaline hematin method.[6] Baseline laboratory parameters included a complete blood count, erythrocyte sedimentation rate, serum ferritin, serum glutamic-pyruvic transaminase, blood urea nitrogen, T_3, T_4, and urinalysis.

Patients were excluded from the study if they had anovulatory cycles (proliferative endometrium) or histologic evidence of pathologic changes in the endometrium (hyperplasia or atypia). They were also excluded if a physical examination revealed that they had an extrauterine disease or palpable leiomyoma. Other criteria for exclusion included a known sensitivity to fenamates, anticoagulant therapy, thyroid dysfunction, hepatic disease, renal disease, or abnormal cervical cytologic findings.

Women who did not have any of these pathophysiologic processes and demonstrated a menstrual blood loss >60 ml in at least one observation cycle were enrolled in the study.

Methodology

Experimental design. Patients who met inclusion criteria were randomly assigned in a double-blind manner to one of two groups by code supplied by an independent biometrics department. The Meclomen group received 100 mg of meclofenamate sodium three times a day during the next two menstrual cycles and were then crossed over to receive an identical-appearing placebo capsule three times a day for the last two menses. The placebo group received the placebo capsules for the first two menses and were then crossed over to receive Meclomen for the last two menses.

Medication was initiated after the onset of menses and continued three times a day for 6 days or until the cessation of menses, whichever came first. During menses, patients collected all tampons and sanitary napkins in a sealed, dry container. The collected material was assayed for menstrual blood loss at the end of each menses.

Patients kept their own diaries, which included the number of days of menstrual flow, medication records, the use of concurrent medications, and the occurrence of side effects or dysmenorrhea.

Patients underwent a history, physical, and evaluation of all the laboratory parameters before the initiation of the study and after the 4-month trial period ended. A complete blood count, platelet count, mean corpuscular volume, and mean corpuscular hemoglobin concentration were also analyzed on the sixth day of the first treatment cycle, the cycle before crossover, and in the final cycle.

Pads and tampons were analyzed for the amount of blood loss at the end of each collection period. The assay was performed as previously described[7] by extraction of blood from pads and tampons by soaking in sodium hydroxide for 24 hours. The blood was then filtered through a Whatman No. 1 filter paper to remove fibers. The measurement of the hemoglobin concentration from the extracted blood was performed by spectrophotometry at an optical density of 546 mm. The concentration of hemoglobin was then compared with the hemoglobin concentration in a known volume of venous blood to calculate the volume of blood loss.[7]

Statistical analysis. The primary measure of effectiveness was based on the ability of the drug to reduce menstrual blood loss. For the baseline analysis of two treatment groups, a one-way analysis of variance was used. The overall significance level was 5% for all statistical evaluations performed.

To compare the overall blood loss and decrease in blood loss from baseline during drug cycles with blood loss during placebo cycles in both groups of patients, a two-treatment four-period crossover analysis of variance was used. This type of analysis was also used to compare pad and tampon use, duration of menstrual flow in days, and severity or occurrence of prostaglandin-related symptoms, such as dysmenorrhea, backache, headache, nauesa, and/or vomiting.

Independent t tests with a Bonferroni correction factor were used to analyze individual comparisons of menstrual blood loss and decreases in blood loss from baseline levels. The overall significance level was adjusted to 1.25% for the evaluations performed.

Subjects were classified into one of three categories to evaluate the percentage distribution of patients who experienced a reduction of blood loss from baseline during Meclomen cycles. A χ^2 test was used for this analysis. The categories were divided into those patients who had a decrease in blood loss from baseline during both Meclomen cycles, those who had a decrease from baseline in one Meclomen cycle, and those who did not have a decrease in blood loss during any Meclomen treatment period.

Study population. Of 95 patients who underwent the screening process, 32 women qualified on the basis of the previously stated criteria and were entered into the study. Of these women, 15 were randomly assigned to the Meclomen-placebo group and 17 to the placebo-Meclomen group. The mean age, body weight, and race distribution were similar in both groups (Table I).

Of the 32 women, three had a past history of anemia and three others had concurrent medical conditions that did not exclude them from the study. The latter three patients included one with hypothyroidism who was taking replacement therapy, another with a history of hypertension that occasionally required treatment

Table I. Characteristics of patients enrolled in each treatment group

	Mean age (yr) (range)	*Mean weight (lb) (range)*	*Race*	
			White	*Black*
Meclomen (n = 15)	36.4 (19-45)	149.3 (108-213)	12	3
Placebo (n = 16)	35.3 (29-43)	164.7 (120-130)	13	3

Table II. Percentage of patients experiencing a decrease in blood loss from baseline*

Drug	*2 cycles (%)*	*1 cycle (%)*	*0 cycles (%)*
Placebo	14 (48)	8 (27)	7 (24)
Meclomen	26 (89.7)	0	3 (10.3)

*$p < 0.0062$.

Table III. Duration of flow and number of sanitary pads and tampons used during drug and placebo cycles

	Days of flow ($\bar{x} \pm SEM$)	*No. pads and tampons ($\bar{x} \pm SEM$)*
Meclomen cycles (n = 58)	4.8 ± 0.20*	15.5 ± 0.9†
Placebo cycles (n = 58)	5.4 ± 0.18	27.6 ± 2.1

*$p < 0.0003$.
†$p < 0.0001$.

Table IV. Evaluation of severity of menstrual symptoms during drug and placebo cycles

Symptom	*Meclomen cycles (n = 58) mean score per cycle*	*Placebo cycles (n = 58) mean score per cycle*
Dysmenorrhea*	0.89*	1.38
Backache†	0.20†	0.50
Headache‡	0.25‡	0.63
Nausea	0.13	0.17
Vomiting	0.0	0.05

N = Number of menstrual episodes: 0 = none, 1 = mild, 2 = moderate, and 3 = severe.
*$p < 0.006$.
†$p < 0.02$.
‡$p < 0.002$.

with hydrochlorothiazide, and one receiving isoniazid (INH) therapy for a positive tuberculosis test.

All patients had regular cycle bleeds with no intermenstrual spotting, 10 (31%) had severe dysmenorrhea, 16 (50%) had moderate dysmenorrhea, and six (18%) are or were dysmenorrheic.

All but four patients had one or more living children, seven (21%) were currently using an intrauterine device for contraception, six (18%) had undergone previous sterilization procedures, and two (15%) used barrier methods. Eleven women either had partners with vasectomies or were not sexually active.

Two patients discontinued the study after the screening phase for personal reasons. Another patient discontinued the study because of gastric distress after one cycle of Meclomen. Twenty-nine women completed the 4-month double-blind trial and are included in the final statistical analysis. The Meclomen-placebo group included 13 patients and the placebo-Meclomen group included 16 patients.

Results

The mean baseline menstrual blood loss was 141 ± 17.5 ml in group 1 and 141.8 ± 26.5 ml in group 2, which did not differ significantly. The menstrual blood loss during the Meclomen treatment periods (69.0 ± 6.3 ml) was significantly less than the blood loss during the baseline screening cycles (141.6 ± 15.9 ml) and the placebo cycles (135.6 ± 11.3) ($p < 0.0001$) (Fig. 1).

The mean blood loss during the trial cycles is seen in Fig. 2 and is graphed sequentially according to cycle treatment period and treatment group. Blood loss was significantly reduced during the Meclomen cycles when compared with the placebo cycles for periods 1, 2, and 3. The mean blood loss during the Meclomen treatment periods ranged from 58.3 ± 11.5 ml in cycle 3 to 81.2 ± 15.8 ml in cycle 1. The mean blood loss during the placebo periods ranged from 117.3 ± 23.5 ml in cycle 4 to 156.6 ± 24.4 ml in cycle 1. There was no demonstrable order effect whether drug or placebo was administered first and no residual effect from previous treatment periods.

The percentage change from baseline is shown in Fig. 3 according to treatment period and group. The overall decrease in blood loss was significantly greater during the Meclomen treatment periods (−48.9 ± 3.7) than during the placebo treatment periods (−9.2 ± 5.3; $p < 0.0001$). Blood loss was significantly reduced during individual treatment periods 1, 2, and 3. The percentage change in blood loss ranged from −42.4% ± 3.0% in cycle 1 to −55.8% ± 8.3% in cycle 3 during Meclomen administration and from

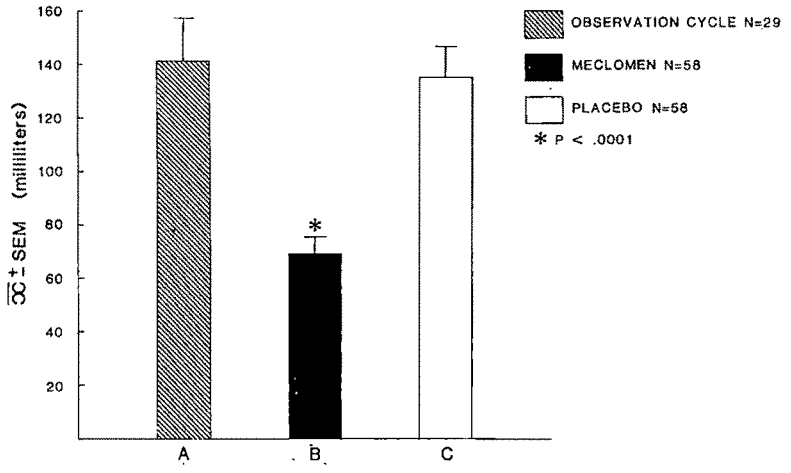


Fig. 1. Menstrual blood loss.

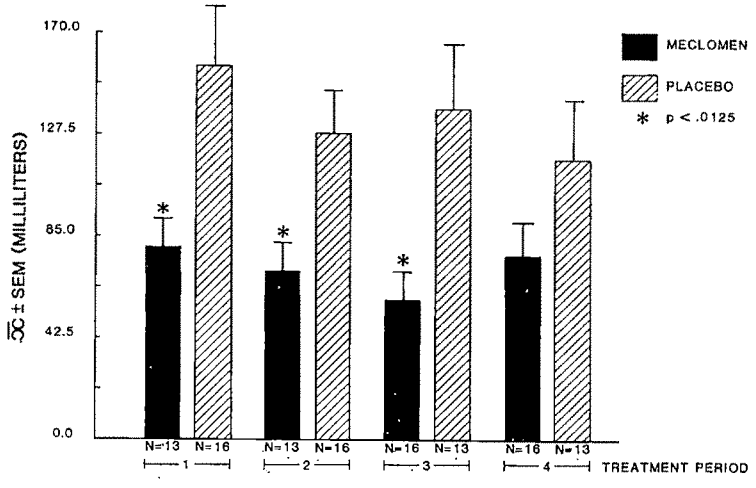


Fig. 2. Menstrual blood loss by treatment period and drug.

−26.0% ± 9.1% in cycle 4 to +5.8% ± 11.9% in cycle 1 during placebo cycles. There was no demonstrable order effect whether drug or placebo was administered first and no residual effect from previous treatment periods.

The number of patients who experienced a decrease in blood loss during both one or no treatment cycles is seen in Table II. There was a statistically significant difference in the Meclomen response pattern when compared with the placebo response pattern.

Pad and tampon use per menstrual cycle and the number of days of menstrual flow are summarized in Table III. During Meclomen cycles, the mean number of pads and tampons used per patient per day was less than the number used in placebo and baseline cycles ($p < 0.0001$). Likewise, it was found that the duration of flow was significantly less during Meclomen cycles (4.8 ± 0.0) when compared with placebo (5.5 ± 0.18) or baseline cycles (6.3 ± 0.41; $p < 0.0003$).

Table V. Median hemoglobin, hematocrit, and ferritin levels in all subjects before and at end of study

	Baseline	*Study termination*
Hemoglobin (gm/dl)	13.2	12.8
Hematocrit (%)	39.4	38.9
Ferritin (ng/ml)	16.0	14.8

The severity of prostaglandin-related symptoms was rated by patients on a daily basis from none = 0 to severe = 3. The results are summarized in Table IV. The severity of dysmenorrhea, backache, and headache while patients took Meclomen was significantly less than

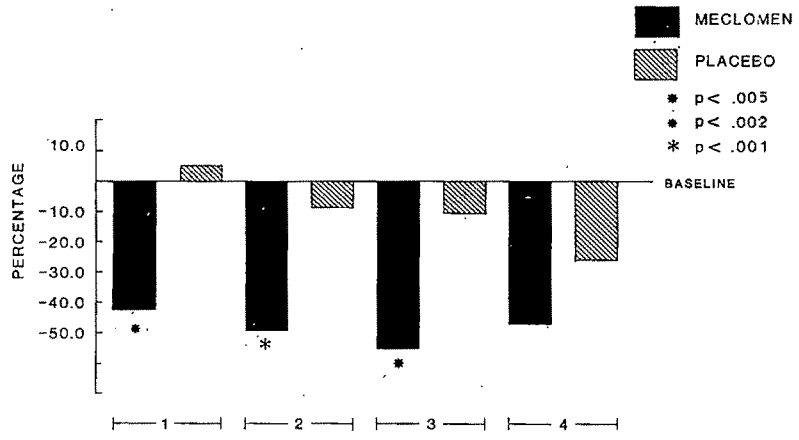


Fig. 3. Percentage change from baseline in blood loss by treatment period and drug.

that while patients took the placebo. There was, however, no difference in nausea or vomiting when ratings during the Meclomen and placebo cycles were compared.

The median hematocrit, hemoglobin, and serum ferritin levels in groups 1 and 2 were similar at the onset of the study. The overall median hematocrit/hemoglobin was 39.35/13.15. These parameters remained unchanged throughout the 4-month study, and levels were not significantly altered after treatment. Likewise, serum ferritin levels were similar at baseline and remained stable throughout the study (Table V).

Five patients experienced side effects while taking Meclomen. Four patients had episodes of nausea and vomiting that may have been related to the drug or prostaglandin release at the onset of menses. One patient had a 3-day episode of epigastric distress after ingestion of the medication on an empty stomach.

Comment

Many women have menorrhagia of unexplained origin. The disorder has been estimated to occur in 2% to 10% of the female population.[7] Although the pathophysiologic mechanisms responsible for the excessive blood have not yet been definitively identified, recent data implicate elevated levels of prostaglandin E_2 and prostacyclin in the endometrium as potential etiologic factors.[8]

Luteal phase endometrial levels of prostaglandins are increased in women with regular but excessively heavy menses when compared with levels in women with normal menses.[2] The increase in prostaglandin E is significantly greater than that for prostaglandin$_{2\alpha}$.[2] Moreover, the follicular phase levels of the endometrial prostaglandins are also elevated unlike those in women with normal menses or dysmenorrhea in whom a cyclic variation is demonstrated.[2] The data imply chronically elevated levels of endometrial prostaglandin E in this subgroup of women. Although peripheral steroid levels do not demonstrate an endocrine disregulation as the cause of menorrhagia, an altered endocrine response of the endometrium may be hypothesized as a causative factor.

Other data that support this theory is found when endometrial receptor profiles are evaluated. Hoffman et al. showed that receptor binding for prostaglandins E and $E_{2\alpha}$ is highest in the fundus of the uterus and correlates with the amount of smooth muscle content found in the sample. When different gynecologic conditions were compared, there was no difference in prostaglandin $E_{2\alpha}$ binding. However, prostaglandin E binding was significantly elevated when uteri from patients with abnormal uterine bleeding are compared with those from patients with dysmenorrhea.[3]

Women using an intrauterine contraceptive device have an increase in menstrual blood loss. In one series, 47.7% of women had a blood loss >80 ml, and all women increased their blood loss by 106% after insertion.[9] Likewise, when the prostaglandin content of the endometrium is evaluated before and after insertion of the intrauterine device, a statistically significant increase in prostaglandin E but not E_2 is found in 11 of the 14 patients evaluated.[10] Mefenamic acid has been shown to reduce intrauterine device–associated menstrual blood loss.[4] In our series, five of the seven women who were intrauterine device users also had a reduction of blood loss when meclofenamate sodium was compared with blood loss during baseline or placebo cycles.

Several pathophysiologic properties of prostaglandins may account for their role in the etiology of menorrhagia. Prostaglandin E has been shown to be a potent vasodilator and an inhibitor of platelet aggregation and adhesiveness.[11, 12]

Likewise, the prostaglandin endoperoxide prostaglandin H_2 is transformed into thromboxane A_2 and prostacyclin (prostaglandin I_2). Prostacyclin, like pros-

taglandin E, causes vasodilatation and also prevents the aggregation of platelets by increasing cyclic adenosine monophosphase. On the other hand, thromboxane results in vasoconstriction and platelet aggregation, which possibly negates the effect of prostacyclin.[11, 12]

However, prostacyclin appears to be the main by-product of arachidonic acid when measured after incubations of endometrium and myometrium.[13] When endometrium from women with normal menses and heavy menses (>50 ml) is incubated with control myometrium, a significantly greater amount of *p* 6-oxy-prostaglandin F_1, the stable metabolite of prostacyclin, is produced.[13] If the in vitro increase in conversion of endoperoxides from the endometrium to 6-oxy-prostaglandin F_1 in the myometrium occurs in a similar fashion in vivo, the excessive formation of prostacyclins may then be implicated as an additive factor in the etiology of menorrhagia.[8]

The anti-inflammatory actions of the prostaglandins are thought to result from the inhibition of prostaglandin E_2 and prostacyclin.[14] The ability of certain prostaglandin inhibitors may also be the result of their ability to reduce the levels of these substances and menstrual blood loss. Meclofenamate sodium has been found to inhibit prostaglandin E_2 formation in bovine seminal vesicles to a significantly greater degree than naproxen, ibuprofen, and benoxaprofen (25→200 × >). The effect was similar to indomethacin.[15] The increased potency of meclofenamate sodium in the reduction of prostaglandin E_2 may account for its efficiency in the reduction of menstrual blood loss.

Overall, the results of this study clearly indicate that meclofenamate sodium decreases the volume of menstrual blood loss in patients with a blood loss >60 ml and no clinically identifiable uterine disease or endocrine abnormality. Twenty-six of the 29 patients evaluated had a reduction in blood loss ranging from 15% to 91% during active drug periods. Therefore it may be anticipated that about 90% of patients will have a good clinical response to treatment with meclofenamate sodium.

Seventeen of the 29 women studied had a blood loss of <80 ml and 12 of the 29 women had a blood loss of <60 ml during both drug administration periods. Three additional patients had a blood loss of <60 ml, and one patient had a blood loss of <80 ml during one of the two medication periods. Halberg et al.[1] showed that although there was some reduction in mean corpuscular hemoglobin concentrations in women with a blood loss >60 ml, the mean peripheral hemoglobin concentrations did not decrease significantly until menstrual blood loss was >80 ml. Therefore it can be anticipated that approximately 60% to 72% of women will not be at risk for anemia with this treatment regimen even without iron supplementation.

The lack of a statistically significant difference in mean blood loss and percentage decrease from baseline between meclofenamate sodium and the placebo during the fourth treatment period is most likely caused by the cycle-to-cycle variation in patients, which has been demonstrated by several investigators.[16] Haynes et al.[16] found that cycle-to-cycle variation was greater in patients with menorrhagia (39 to 271 ml) than in women with normal menses. This factor most likely influenced the fourth-period placebo response and the difference in the statistical evaluation for this treatment period.

Three of the 29 patients evaluated in our study did not demonstrate a reduction in blood loss when compared with baseline measurements. All three patients used an intrauterine device for contraception during this study. The resistance of some intrauterine device users to treatment may be related to the importance of endometrial fibrinolysis in intrauterine device–associated menorrhagia as evidenced by the effective treatment with a fibrinolysis inhibitor (tranexamic acid).[11]

In clinical practice, it is not feasible to measure menstrual blood loss to determine the efficacy of the medication. However, in our study, the number of days of flow and pads and/or tampons used was significantly decreased during the medication periods when blood loss was reduced. Some of these indicators of blood loss have been found to correlate with actual blood loss by previous studies but not consistently. These parameters may be used as soft indicators of blood loss, and if combined with the patient's subjective assessment and serum hemoglobin levels, it is feasible to evaluate the effect of meclofenamate sodium on blood loss and differentiate those patients who may benefit from the medication from those who might warrant further diagnostic and/or therapeutic measures.

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Configuration of the prepubertal hymen

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Anatomically descriptive terms are required when one is asked to comment on the hymen of the sexually abused prepubertal child. Action descriptive terms such as virginal or ruptured relay no objective information. Biologic hymenal configurations can be loosely categorized by the amount and distribution of hymenal tissue surrounding the vaginal introitus: The terms fimbriated, circumferential, and posterior rim are proposed. Descriptions of 124 prepubertal hymens are presented, with discussion focusing on biologic configurations and the impact of trauma and hormones. (Am J Obstet Gynecol 1987;157:950-6.)

Key words: Hymen, sexual abuse, prepubertal, biologic configurations

Abnormal variations in hymenal configurations such as the imperforate, the cribriform, the septate, and the microperforate hymen are well documented.[1, 2] Variations in normal hymenal configuration have also been noted[1, 3-6] and can be loosely categorized by the amount and distribution of hymenal tissue surrounding the vaginal introitus.

The most abundant amount of tissue appears as a redundant, gathered skirt of hymenal tissue surrounding the vaginal introitus. It has been variously described as "irregular, festonne or dentelle or divided into multiple fingers"[3] or as "fimbriated or irregularly notched in outline."[6]

An intermediate amount of tissue exists when the arrangement of hymenal tissue appears smooth, circumscribing the vaginal introitus without folds and with an annular opening. This type has been called a "ring, generally broadest posteriorly,"[4] "annular,"[7] or "annular with oval central opening."[6]

The hymenal configuration with the least amount of tissue present is arranged in a semilunar fashion as a rim of tissue that extends variously from 1 to 2 o'clock, posteriorly around to 10 or 11 o'clock (anterior is 12 o'clock and posterior is 6 o'clock). This hymenal configuration has no or minimal tissue visualized anteriorly and has been described as "a semilunar fold, with its concave margin turned towards the pubes,"[4] "crescentic in appearance,"[6] "congenitally incomplete,"[8] "narrow rim,"[1] and "semilunar or falciform."[3]

Understanding normal hymenal configurations is crucial to the clinician who is asked to evaluate the genitalia of children who may have been sexually abused. Only by knowing normal anatomy can one make meaningful comments and descriptions about alterations that may have occurred as a result of physical trauma. The current practice of placing emphasis on the diameter of the vaginal or hymenal orifice as an indicator of normality or evidence of sexual abuse in the prepubertal child[1, 8-12] should be discouraged when used as an isolated parameter. Normal variations in the

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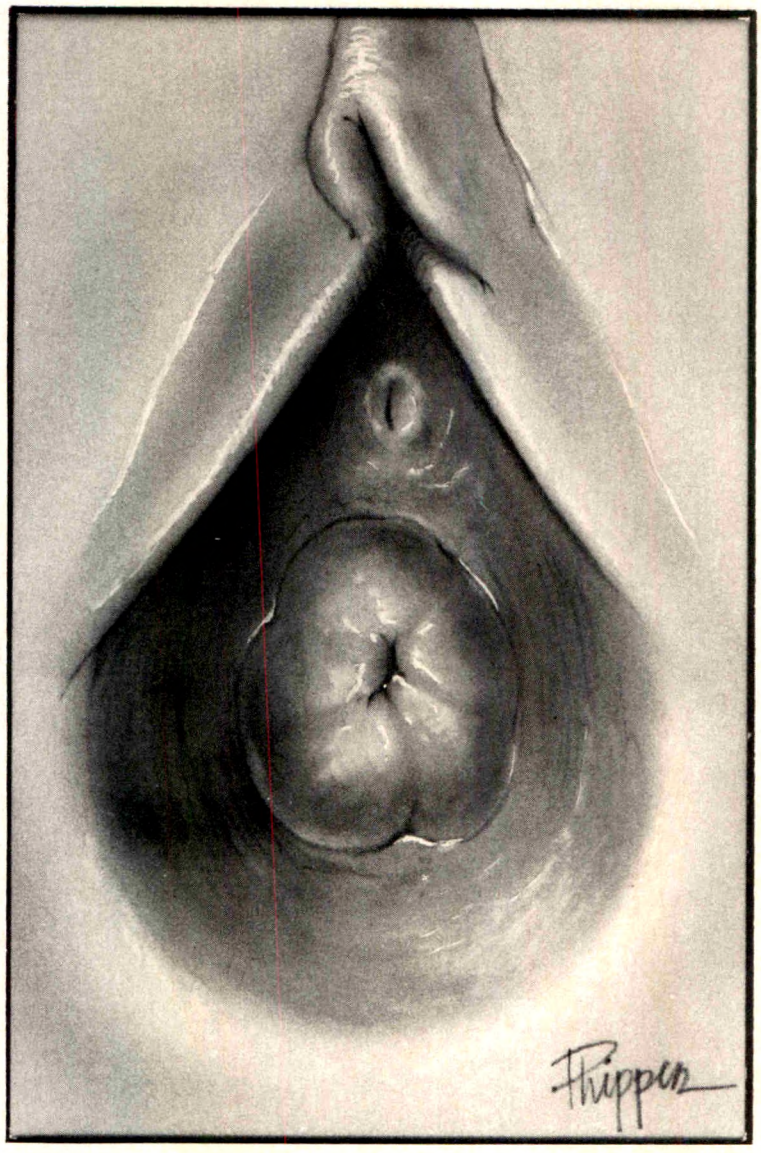


Fig. 1. Fimbriated hymen.

amount and distribution of hymenal tissue will influence the diameter of the vaginal or hymenal orifice.

The hymenal configuration as described in the medical records of 124 prepubertal females is the basis of this article.

Methodology

Anatomic hymenal descriptions were recorded in the medical records of 124 premenarchal female children ages 9 weeks to 10 years. During a 2-year period I saw these patients for a gynecologic examination. The following variables were analyzed: the hymenal configuration, age, race, source of and reason for referral, and any other gross perineal anatomic variation.

For purposes of this study, fimbriated hymens were redundant, gathered skirts of hymenal tissue with scalloped rims (Fig. 1); circumferential hymens were smooth unfolded skirts of hymenal tissue with uniform annular rims (Fig. 2); and posterior rims were crescentic smooth folds of tissue arranged from 2 o'clock through 11 o'clock around the introitus with minimal or no tissue visualized anteriorly (Fig. 3).

Table I. Age and ethnic distribution

Age (yr)	*White n (%)*	*Black n (%)*	*Hispanic n (%)*
Under 2	5 (6.8)	1 (3.1)	0 (0.0)
2-4	31 (42.5)	8 (25.0)	12 (63.2)
5-7	22 (30.1)	11 (34.4)	2 (10.5)
8-10	15 (20.5)	12 (37.5)	5 (26.3)
Total	73	32	19

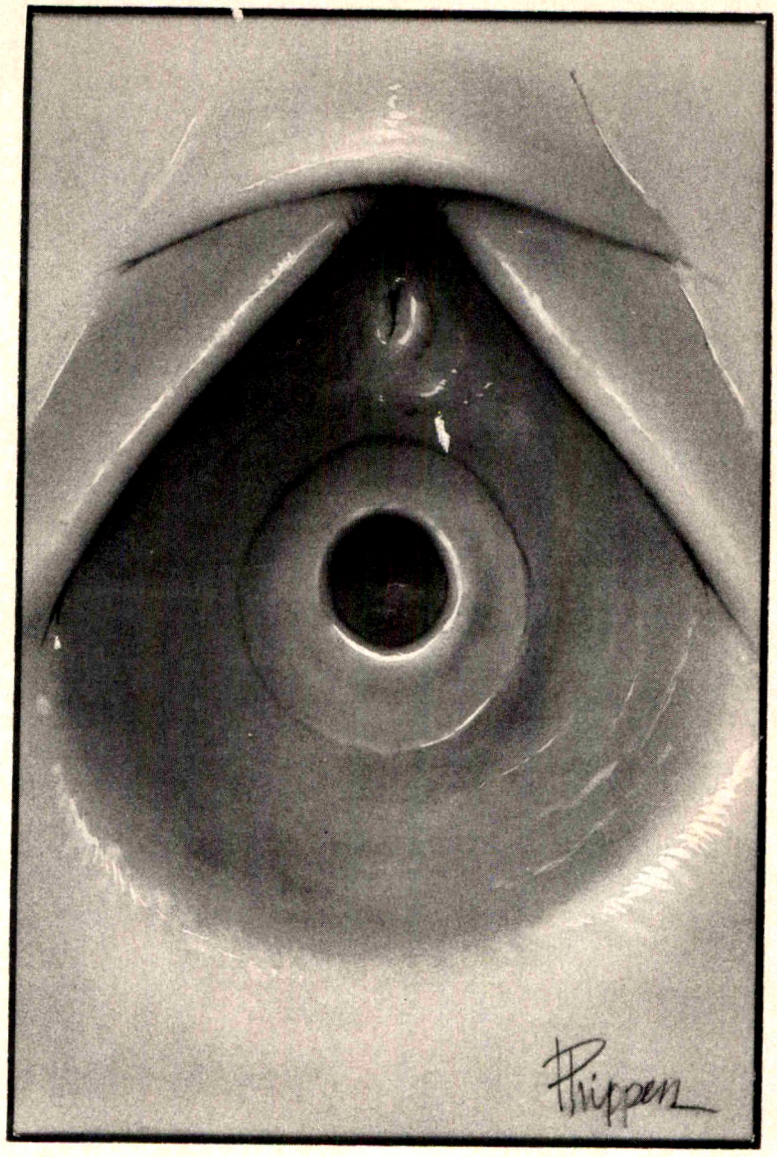


Fig. 2. Circumferential hymen.

Table II. Characteristics of patient visits by age

	Referral pattern (%)			*Reason for evaluation (%)*			
Age (yr)	*Community agency*	*Parent*	*Patient's physician*	*STD*	*PSA*	*VV*	*Misc*
Under 2	3	0	3	0	4	0	2
2-4	16	6	29	0	29	11	11
5-7	11	2	22	1	15	12	7
8-10	13	3	16	3	18	8	3
Total	43 (34.7)	11 (8.9)	70 (56.5)	4 (3.2)	66 (53.2)	31 (25.0)	23 (18.5)

STD = Sexually transmitted disease; PSA = possible sexual abuse/assault; VV = vulvovaginitis; Misc = miscellaneous.

Results

The results of age and race distribution are noted in Table I. The white and Hispanic patients were slightly younger than the black patients, although very few patients under the age of 2 years were seen. Referrals came from other physicians, parental initiation, and community children's protective and investigative agencies. Analysis of referral patterns and the reason

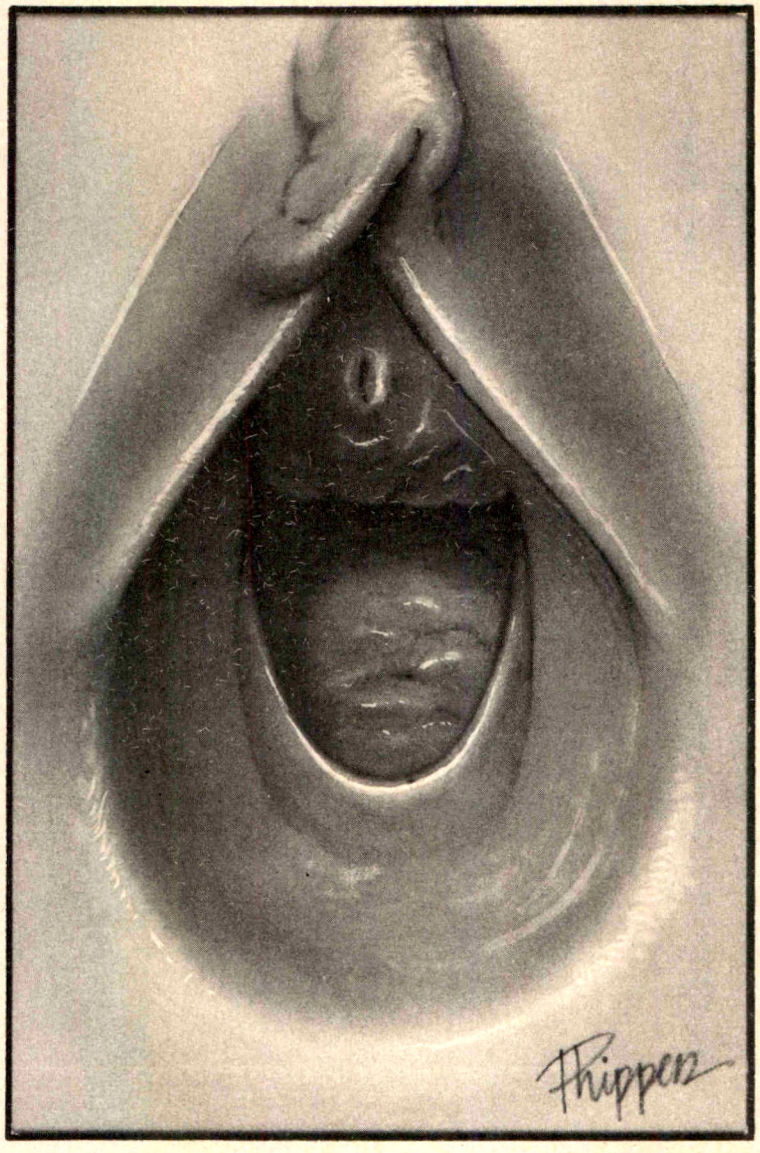


Fig. 3. Posterior rim hymen.

Table III. Distribution of hymenal configurations by age

Age (yr)	*Fimbriated n (%)*	*Circumferential n (%)*	*Posterior rims n (%)*	*Other* n (%)*
Under 2	1 (4.0)	1 (2.9)	3 (5.4)	1 (11.1)
2-4	13 (52.0)	20 (58.8)	16 (28.6)	2 (22.2)
5-7	3 (12.0)	9 (26.5)	21 (37.5)	2 (22.2)
8-10	8 (32.0)	4 (11.8)	16 (28.6)	4 (44.4)
Total	25 (20.0)	34 (27.0)	56 (45.0)	9 (7.0)

*One case of microperforate, two cases of imperforate, six cases of absent or remnants only. Absent or remnants: two cases ages 2 to 4 years, 1 case age 5 to 7 years, and three cases ages 8 to 10 years.

for the visit (Table II) reveals that most patients were referred by other physicians for an evaluation of possible sexual abuse or assault. Vulvovaginitis accounted for another large group of patients. The miscellaneous category included a broad range of entities including the following: labial agglutination, imperforate hymen, ectopic anus, accidental trauma to the perineum, urethral prolapse, premature telarche, and diethylstilbesterol exposure.

In this series (Table III), 25 hymens were classified



Fig. 4. Example of a fimbriated hymen in a 10-year-old child.

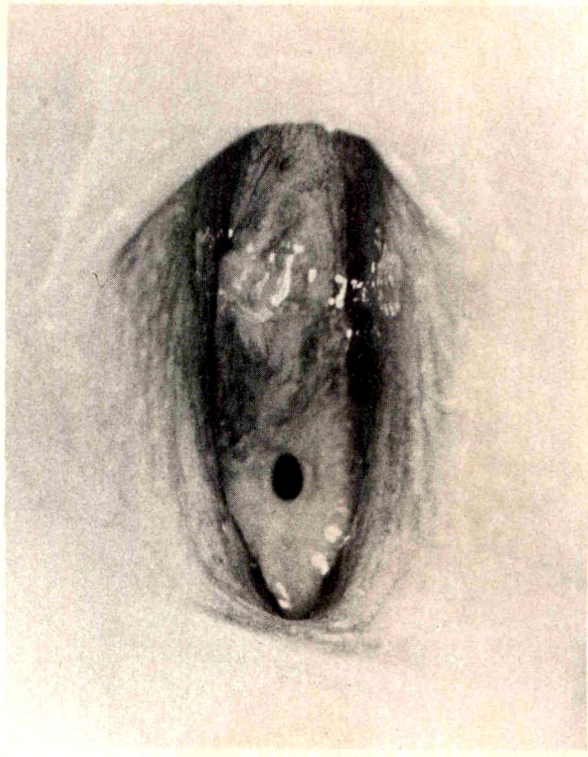


Fig. 5. Example of a circumferential hymen in a 3-year-old girl.

as fimbriated (Fig. 4), 34 as circumferential (Fig. 5), 56 as posterior rim (Fig. 6), and nine as "other." The fimbriated and circumferential configurations were more prevalent in patients less than 4 years of age. There was a fairly even age distribution of those children with posterior rims. In the "other" category, six children were further subcategorized into having absent or remnants of hymen only (Fig. 7). Five of these six children had substantive evidence of sexual abuse; the sixth child, whose grandfather had been accused of molesting other children, was never adequately investigated and was lost to follow-up.

One of the cases listed under fimbriated was a 10-year-old white girl who was at Tanner breast Stage II and pubic hair Stage I when she was violently raped. She underwent surgical repair of a large vaginal laceration and a second-degree laceration of her posterior forchette. On completion of the surgery, the patient was noted to have a prominent rosette of hymenal tissue (fimbriated) surrounding the vaginal introitus. When seen in follow-up 2 weeks later, only small remnants of hymenal tissue remained.

Definite breaks or significant alterations in the hymenal skirt of tissue were noted in 31 children. When reasons for examination were grouped according to those with a strong possibility of being sexually abused (sexually transmitted disease/possible sexual abuse, n = 70) and all others (vulvovaginitis/miscellaneous, n = 54), the suspect sexually abused children were 3.5 times as likely to have a broken and/or altered hymen than the other group. Although all types of hymenal configuration are represented among the children with recorded breaks or alterations, there is a definite trend for breaks to be noticed on hymens with scantier amounts of tissue.

Comment

The hymen is a small organ that surrounds the opening of the vaginal introitus. It is made up of elastic and collagenous connective tissue whose surfaces are covered by stratified squamous epithelium.[3] Its importance to mankind has been predominantly cultural. In the last decade physicians have needed more objective information about the hymen as they have attempted to evaluate their female patients for possible sexual abuse or assault.

The distensibility of the vaginal orifice and its adjacent organs, when under the influence of adult levels of female hormones, makes it difficult for physicians who are requested to testify about the "virginity" of a certain patient.[8] On the other hand, when the hymen is distended by an object as large as an infant's head in the process of childbirth, readily recognizable changes in the hymen occur as it shrivels into what are called the "myrtiform caruncles."[5] Thus we know from observations of the estrogenized hymen that two factors

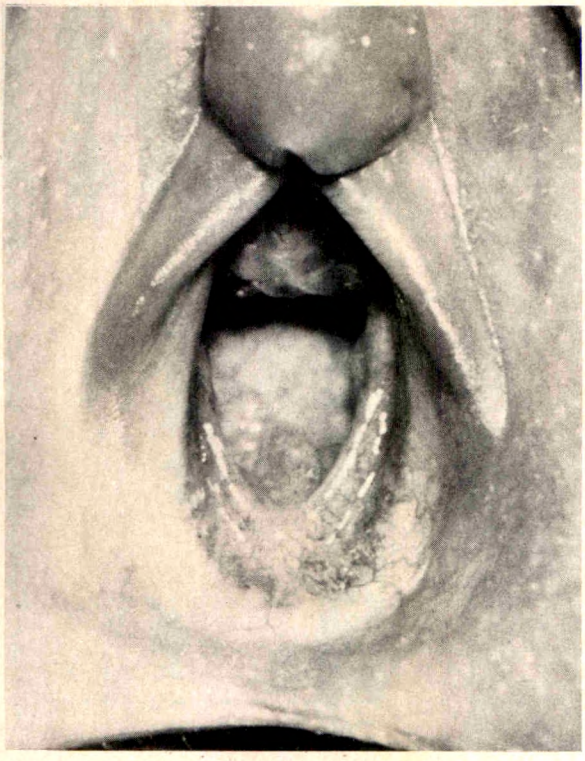


Fig. 6. Example of a posterior rim in a 3-year-old child.

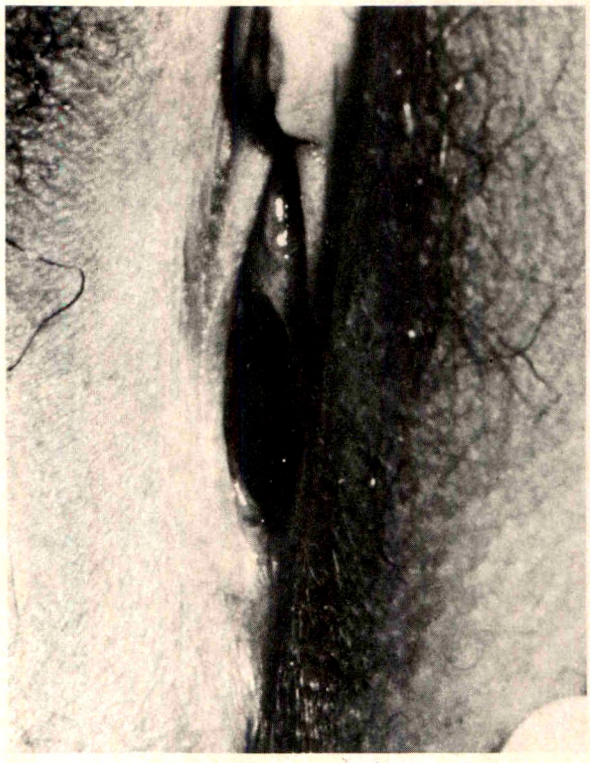


Fig. 7. Example of remnants only in a 7-year-old girl.

influence the hymen configuration: hormones and trauma.

The question must be raised about whether the six children in this series, who were noted to have remnants only, and the one 10-year-old girl who is listed as fimbriated but who had remnants only 2 weeks after surgery, are not in fact the prepubertal equivalent of myrtiform caruncles.

Longitudinal studies will better define the impact of hormonal changes on the hymen. It has been noted[11] that it appears to be less traumatic to examine the vagina of a child less than 2 years old than to perform the same procedure on an older child. This most likely reflects the influence of the maternal estrogen effect on the younger child's hymen, which makes it pink white and thicker as opposed to the unestrogenized hymen, which is redder and much thinner.

A biologic hymen configuration most likely occurs before birth. The hymen is formed at the site where the solid mullerian ducts join the urogenital sinus.[13] It appears that the biologic mechanism for opening the hymen occurs anteriorly. All microperforations are anterior. It has been noted in this series as well as by Gray[4] that there is a tendency for the annular opening of the circumferential hymen to be anteriorly placed, and the posterior rim has minimal or no visible membrane anteriorly. This anterior locus of activity raises the question about whether the perforations of the hymenal membrane are made by the same biologic process that recanalizes the vagina.

The distribution of hymenal configuration by age and type as reported in this article *cannot* be considered a normal population distribution; this study was performed on patients with known or suspected problems. This effort was undertaken to encourage clinicians to develop skills in objective descriptions of the hymen. Action descriptive terms such as virginal, marital, perforated, ruptured, and penetrated relay no objective information. Anatomically descriptive terms are required. I propose three categories based on the amount and distribution of the hymenal tissue: fimbriated, circumferential, and posterior rim.

Configuration is the parameter emphasized in this article. Determining the basic biologic configuration of the prepubertal hymen is seen as a preliminary step before studies can be undertaken to determine changes caused by physical or infectious trauma or hormones. Does a particular biologic hymenal configuration change over time? In this small study, which covered a 2-year period, no changes occurred in the few children who were seen in subsequent visits except for the one 10-year-old rape victim whose fimbriated hymen became remnants only. On the other hand, overall there were more fimbriated and circumferential hymens (i.e.,

a more abundant amount of hymenal tissue) in children less than 4 years of age.

Is there any significance between a high and a low posterior rim? Would gradual, coercive dilation of a child's hymen convert it from a high to a low posterior rim or from a fimbriated or circumferential to a posterior rim?

These and other questions cannot be answered until clinicians learn to identify and discern the anatomic detail of prepubertal hymens and undertake prospective studies.

I would like to thank Claudia Kozinetz for her assistance in compiling the tables.

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Suppression of lymphocyte reactivity in vitro by supernatants of explants of human endometrium

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To clarify the possible immunologic functions of the endometrium before implantation, supernatants from explant cultures of human endometrial tissues were examined for effects on mixed lymphocyte reaction and phytohemagglutinin M–stimulated cultures. Supernatants obtained from both proliferative and secretory phase endometria showed significant dose-related suppressive effects; the suppressive activity of secretory phase endometria was higher than that of proliferative phase endometria. On the other hand, no inhibitory effect was identified in supernatants from similar cultures of peritoneum and omentum, while supernatants of fallopian tubes showed slight suppressive activity. These results suggest the existence of soluble nonspecific immunosuppressive factor(s) released from human endometrium (especially during the secretory phase), and imply that these factor(s) may exert an important role in protecting the fertilized ovum from maternal rejection. (Am J Obstet Gynecol 1987;157:956-63.)

Key words: Endometrial supernatant, mixed lymphocyte reaction, phytohemagglutinin M, immunosuppression

During the last decade much attention has been focused on local regulation of the maternal immune response in the uterus toward the explanation of how the conceptus avoids immunologic rejection. The uterus is a unique organ of considerable immunologic interest; not only must it deal with constant exposure to the external environment, it must also serve as the recipient site for the natural transplantation of genetically alien spermatozoa and conceptuses, without developing destructive immune responses. A major part of these functions is borne by the uterine endometrium, and

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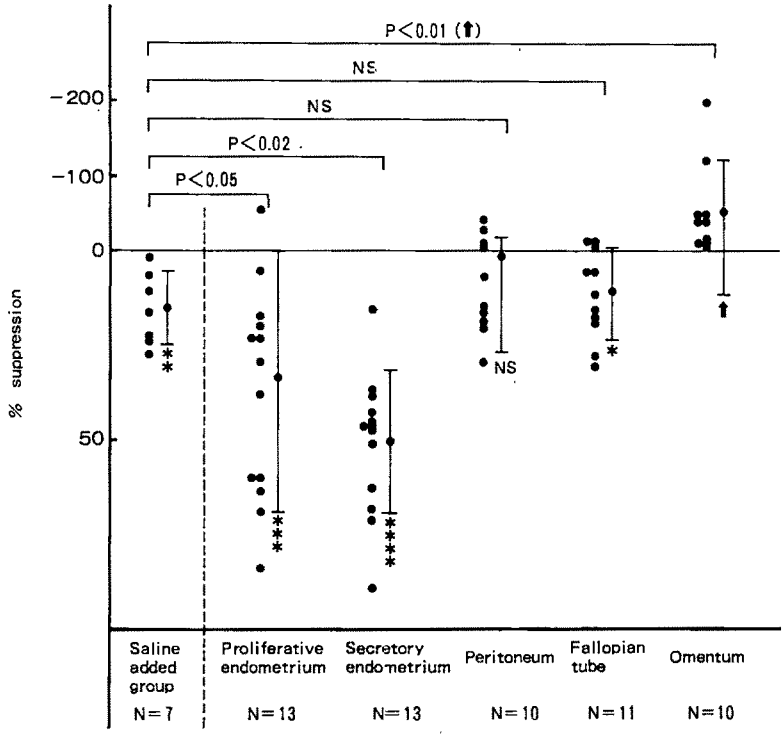


Fig. 1. Effect of tissue culture supernatants on the mixed lymphocyte reaction. Results are expressed as percent suppression in comparison with control cultures (peripheral blood lymphocytes plus Trowell's T8 medium plus allogeneic cells but without supernatant). Each *point* represents the mean percent suppression of triplicate incubations, and *vertical bars* represent the mean ± SD of each group. Statistical significance was calculated by paired Student's *t* test: * = $p < 0.05$; ** = $p < 0.02$; *** = $p < 0.01$; **** = $p < 0.001$; NS = not significant. ↑ = Enhanced effect.

several recent reports have suggested the important immunoprotective role of decidua,[1, 2] the end product of a hormonally induced transformation of the endometrium. However, it is not known whether nondecidualized endometrium also possesses immunoregulatory properties. To clarify the immunologic role of the endometrium, we examined the effects of supernatants obtained from tissue culture of human endometrium on mixed lymphocyte reaction and phytohemagglutinin M–stimulated lymphocyte activation.

Material and methods

Tissue cultures. Human endometrial tissues were obtained at hysterectomy in 28 women aged from 35 to 45 years without malignancy and who had not received hormone therapy for at least two menstrual cycles before the operation. The tissues were washed twice in cold Hanks' balanced salt solution, once in Trowell's T8 medium (Gibco Laboratories, Grand Island, New York), and then cut into pieces of approximately 1 to 2 mm^3. Five to seven pieces of endometrial tissues (100 to 200 mg wet weight) were placed on a sterile stainless mesh in a six-well Petri dish (Falcon 3046, Becton-Dickinson & Co., Oxnard, California) containing 2 ml of Trowell's T8 medium supplemented with 10% heat-inactivated fetal calf serum (Gibco), 100 IU/ml of penicillin G, and 100 μg/ml of streptomycin sulfate. A portion of the tissues was prepared for histologic examination and determination of the menstrual cycle. The tissues were incubated in a humidified atmosphere of 95% air and 5% carbon dioxide at 37° C for 1 hour. Next, the culture medium was discarded to remove cell debris and toxic products, and 2 ml of new culture medium was added. After an additional culture for 12, 24, 48, or 72 hours, the culture medium was centrifuged, collected, and stored at −20° C. Trowell's T8 medium contained 10% fetal calf serum and antibiotics were incubated in the same manner as the medium control. To serve as controls, tissue specimens from the fallopian tube, peritoneum, and omentum from 13 of these patients were treated in the same manner.

Preparation of lymphocytes. Peripheral blood lymphocytes were obtained from healthy male volunteers by Ficoll-Hypaque centrifugation. The separated cells were washed three times in RPMI-1640 medium and resuspended in the same medium (Gibco) supplemented with 10% fetal calf serum and antibiotics as above.

Mixed lymphocyte reaction. Peripheral blood lym-

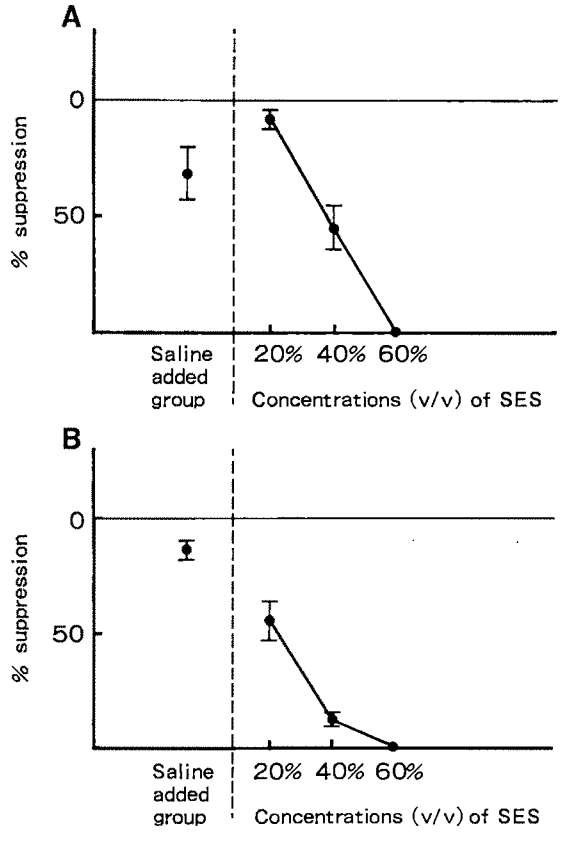


Fig. 2. Dose-dependent suppression by secretory phase endometrium *(SES)* on mixed lymphocyte reaction **(A)** and phytohemagglutinin–induced lymphocyte stimulation **(B)**. Each well contained peripheral blood lymphocytes in a density of 2×10^5/well **(A)** or in a concentration of 1×10^6/ml **(B)** with various concentrations (vol/vol) of secretory phase endometria. Results are expressed as percent suppression in comparison with control cultures (peripheral blood lymphocytes plus Trowell's T8 medium plus allogeneic cells or phytohemagglutinin but without supernatant). Each value is the mean ± SD of three replications.

phocytes suspensions from two unrelated volunteers were added in equal proportions (70 μl) to U-bottomed 96-well microculture plates (Corning Glass Works, Corning, New York) at a cell density of 2×10^5/well. The stored supernatants (from 48-hour culture) and control culture medium were thawed, sterilized by passing through a 0.22 μm Millex-GV filter unit (Millipore Co., Bedford, Massachusetts) just before use, and added to wells at a final concentration of 40% (vol/vol). Cultures were performed at 37° C in a humidified atmosphere of 5% carbon dioxide in air for 6 days, and 1 μCi of ^{3}H-thymidine (2 Ci/mmol, Amersham, Buckinghamshire, United Kingdom) was added to each well 20 to 24 hours before the end of the culture. The contents of each well were then harvested on filter papers with the use of a semiautomatic multiple cell harvester (Labo Science Co., Tokyo, Japan) and dried, and the radioactivity was counted in a liquid scintillation counter (Aloka Co., Tokyo, Japan) for 1 minute.

Phytohemagglutinin M–stimulation culture. Peripheral blood lymphocytes (1×10^6 cells/ml) and 10 μl/ml of phytohemagglutinin M (Difco Laboratories, Detroit, Michigan) were incubated in U-bottomed 96-well microculture plates at 37° C in a humidified atmosphere of 5% carbon dioxide in air. Preliminary experiments had shown that this dose of phytohemagglutinin M provided optimal lymphocyte responses under the same culture conditions. Supernatants or culture medium alone was added as described above to give a total volume of 250 μl/well. After 96 hours of culture including pulsation with 1 μCi/ml of tritiated thymidine for the final 6 to 8 hours, lymphocytes were harvested and counted as described above.

Viability of lymphocytes. The viability of lymphocytes at the end of mixed lymphocyte reaction and phytohemagglutinin M–stimulation cultures was >90% as assessed by trypan blue exclusion.

Calculation of results. All lymphocyte cultures were performed in triplicate, and the mean and standard deviation of the counts per minute (cpm) were calculated. Percent suppression compared with control cultures (peripheral blood lymphocytes + Trowell's T8 medium + phytohemagglutinin M or allogeneic cells but without supernatant) was calculated according to the formula:

$$\% \text{ Suppression} = \left(1 - \frac{\text{cpm in cultures with supernatant}}{\text{cpm in control cultures}}\right) \times 100$$

Statistical significance was examined by paired Student's *t* test.

Results

Effect of supernatants on mixed lymphocyte reaction. As seen in Fig. 1 and Table I, the mixed lymphocyte reaction was significantly suppressed by the addition of both secretory phase endometria (all 13 cases) and proliferative phase endometria (12 of 13 cases) as compared with control cultures ($p < 0.001$ and $p < 0.005$, respectively), with the suppressive activity of secretory phase endometria (mean 50% ± 19%) higher than that of proliferative phase endometria (mean 33% ± 36%). Most of the proliferative phase endometria samples (12 cases) were obtained from the late proliferative phase (cycle day 15 ± 2) and showed a mean suppression of 35% ± 37%. In contrast, one proliferative phase endometria sample from the early phase (cycle day 7) showed no significant suppressive effect, indicating that the suppressive activity gradually increased as the cycle progressed. On the other hand, supernatants of peritoneum and omentum had no sup-

Table I. Outcome of suppression experiments on mixed lymphocyte reaction

Experimental materials	*No. of cases*	*Age (yr)*	*Cycle days*	*Suppressive activity*	*No. showing suppression*	*Paired t test*	
						Compared with control	*Compared with saline solution group*
Saline solution	7			15% ± 10%	7	$p < 0.02$	
Proliferative endometrium	13	40 ± 3	14 ± 3	33% ± 36%	12	$p < 0.005$	$p < 0.05$
Secretory endometrium	13	40 ± 3	28 ± 14	50% ± 19%	13	$p < 0.001$	$p < 0.02$
Fallopian tube	11	42 ± 3		10% ± 13%	8	$p < 0.025$	NS
Peritoneum	10	40 ± 2		1% ± 25%	6	NS	NS
Omentum	10	40 ± 3		−37% ± 68%	0	$p < 0.01$*	$p < 0.01$*

Data are mean ± SD. NS = Not significant.
*Enhanced effect.

Table II. Outcome of suppression experiments on phytohemagglutinin M–induced lymphocyte reactivity

Experimental materials	*No. of cases*	*Age (yr)*	*Cycle days*	*Suppressive activity*	*No. showing suppression*	*Paired t test*	
						Compared with control	*Compared with saline solution group*
Saline solution	9			12% ± 13%	8	$p < 0.05$	
Proliferative endometrium	11	40 ± 4	14 ± 3	45% ± 26%	10	$p < 0.005$	$p < 0.005$
Secretory endometrium	17	41 ± 3	27 ± 3	60% ± 17%	17	$p < 0.001$	$p < 0.001$
Fallopian tube	7	41 ± 2		21% ± 17%	7	$p < 0.05$	NS
Omentum	13	41 ± 3		4% ± 32%	7	NS	NS

Data are mean ± SD. NS = Not significant.

pressive effect, while eight of 11 cases of tubal supernatants showed significant suppression when compared with the control cultures ($p < 0.025$).

The degree of suppression caused by endometrial supernatant was dose related. As shown in Fig. 2, suppressive activity increased as the concentration of secretory phase endometria increased, and at a final concentration of 60% (vol/vol) the lymphocyte response was completely abolished. Fig. 3 shows a dose-dependent curve with a constant concentration of secretory phase endometria (40% vol/vol) with decreasing cell numbers. The suppressive activity was progressively accentuated by decreasing the numbers of cells.

Experiments in which saline solution instead of supernatant was added were also carried out to determine whether the culture supernatants were depleted of nutrients. As shown in Fig. 1 and Table I, lymphocyte responsiveness was significantly ($p < 0.02$) reduced by the addition of saline solution (40% vol/vol of final concentration), with a mean suppression of 15% ± 10% as compared with control cultures. However, the suppressive activity of both secretory and proliferative phase endometria was still significantly greater than that of the saline solution ($p < 0.02$ and $p < 0.05$, respectively). The supernatants obtained from other tissues, including fallopian tubes, caused no significant suppression in comparison with the group that received saline solution.

Effect of supernatants on phytohemagglutinin M-induced lymphocyte reactivity. Both secretory (all 17 cases) and proliferative (10 of 11 cases) phase samples showed significant suppressive effects on phytohemagglutinin M–induced lymphocyte proliferation as compared with control cultures ($p < 0.001$ and $p < 0.005$, respectively; Fig. 4; Table II). The mean suppression of lymphoproliferation was higher in secretory phase endometria (60% ± 17%) than in proliferative phase endometria (45% ± 26%), but the difference was not statistically significant. One proliferative phase endometria sample from the early phase (cycle day 7) showed no significant suppressive effect, and other 10 proliferative phase endometria samples from the late phase (cycle day 15 ± 3) showed significant suppression ($p < 0.025$). The inhibitory effect was dose dependent, similar to that seen in mixed lymphocyte reaction experiments (Fig. 2, *B*). In contrast, the supernatants obtained from omentum (13 cases) showed no suppression, but supernatants from fallopian tubes (seven cases) showed significant suppression in comparison with control cultures ($p < 0.05$). The addition of saline solution to phytohemagglutinin M–stimulation culture showed significant inhibition

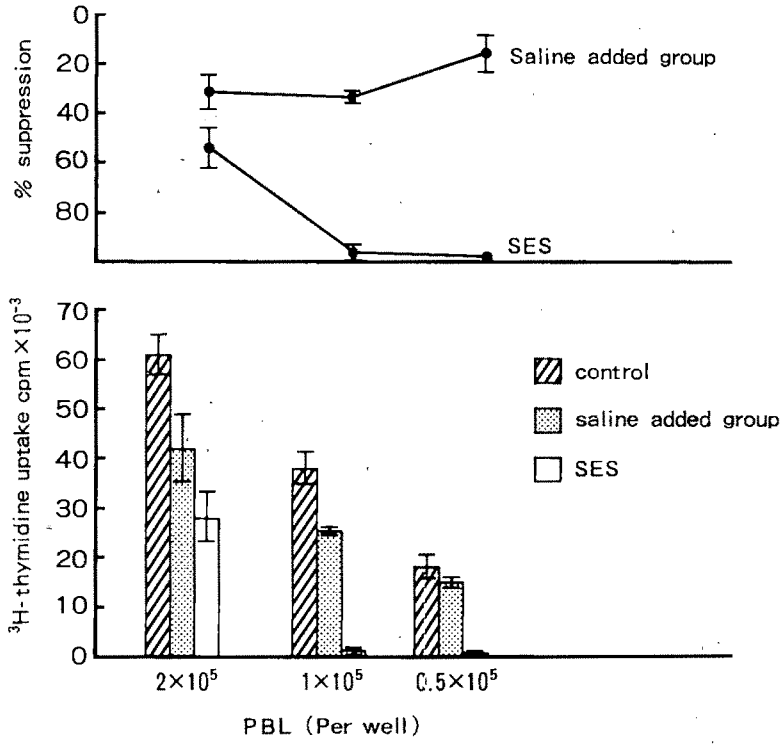


Fig. 3. Dose-dependent suppression of secretory phase endometria *(SES)* of mixed lymphocyte reaction. Each well contained a constant volume of secretory phase endometria (40%) with varying concentrations of peripheral blood lymphocytes *(PBL)*. Results are expressed as percent suppression in comparison with control cultures (peripheral blood lymphocytes plus Trowell's T8 medium plus allogeneic cells but without supernatant) and as counts per minute. Each value is the mean ± SD of three replications.

(mean 12% ± 13%) as compared with control cultures ($p < 0.05$). However, the levels of suppression caused by secretory and proliferative phase endometria were still significantly higher than that of the saline solution group ($p < 0.001$ and $p < 0.005$, respectively). On the other hand, the supernatants from fallopian tubes and omentum showed no significant suppression when compared with the saline solution group (Fig. 4; Table II).

In other experiments, serial dilutions of phytohemagglutinin M were added to the cultures (final concentration 10, 20, and 40 μl/ml) with a constant dose of endometrial supernatants. As shown in Fig. 5, the activation of lymphocytes was stimulated by phytohemagglutinin M, but increased concentrations of phytohemagglutinin M did not further enhance the reactivity of lymphocytes.

Effect of duration of endometrial culture. Supernatants were removed from cultures of human endometria at the times indicated (from 12, 24, 48, and 72 hours) and were tested. Endometrial supernatants from 12-hour culture had little or no suppressive effect on lymphocyte reactivity to phytohemagglutinin M and the mixed lymphocyte reaction (Fig. 6). The levels of suppression were progressively accentuated by increasing the culture times, and were maximum at 48 hours. However, a further 24-hour culture (total 72 hours) had no additional effect, suggesting that the major portion of the suppressive factor(s) was released 48 hours after the start of culture.

Comment

Our present study demonstrates that the supernatants obtained from explant cultures of both proliferative and secretory phase endometria showed marked suppression of the unrelated adult lymphocyte response to phytohemagglutinin M stimulation and the mixed lymphocyte reaction. The suppression of lymphoproliferation by the supernatant of secretory phase endometrium was higher than that of the proliferative phase sample in both mixed lymphocyte reaction- and phytohemagglutinin M-mediated cultures. The inhibitory effect of endometrial supernatant was dose related, nonspecific, and more likely due to the presence of suppressive factor(s) rather than the nutritional depletion, because under poor nutritional culture conditions, the lymphocyte reactivity was enhanced by decreasing the cell numbers (as seen in the group that

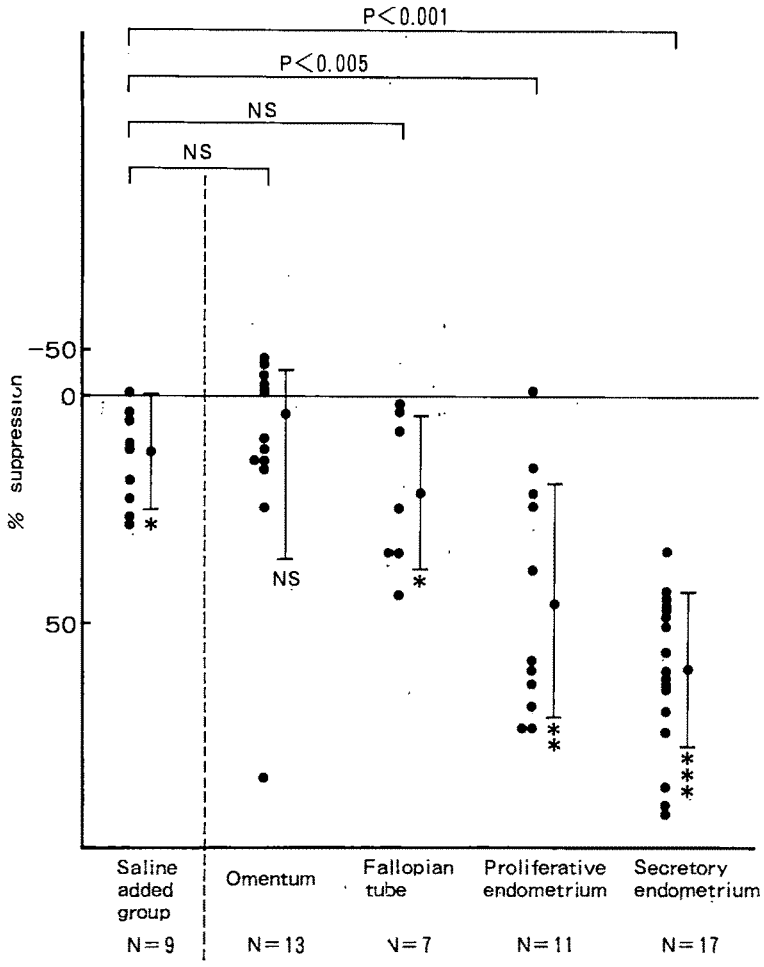


Fig. 4. Effect of tissue culture supernatants on phytohemagglutinin-mediated lymphocyte reactivity. Results are expressed as percent suppression in relation to control cultures (peripheral blood lymphocytes plus Trowell's T8 medium plus phytohemagglutinin but without supernatant). Each *point* represents the mean percent suppression of triplicate incubations, and *vertical bars* represent the mean ± SD of each group. Statistical significance was calculated by paired Student's *t* test as follows: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = not significant.

had saline solution added [Fig. 3]). In the presence of endometrial supernatant, however, lymphocyte proliferation was progressively inhibited by decreasing the cell numbers. Supernatants obtained from the omentum and peritoneum had no suppressive activity. Supernatants from fallopian tubes showed some suppressive activity in comparison with control medium (Trowell's T8 medium), but the level of inhibition was similar to that in the saline solution group. These results suggest that human endometrium releases a soluble suppressive factor that is capable of afferent blockade of the immune response, as the mixed lymphocyte reaction is considered an in vitro analogue of the afferent arm of immune response.

The highest values of progesterone concentrations in both secretory and proliferative phase endometria samples were 3 and 0.5 ng/ml, respectively. These concentrations were too low to inhibit immune reactivity, because several reports[9] and our preliminary study revealed that the minimum effective concentration of progesterone for such suppression was in the range of micrograms. The suppressive effects of mixed lymphocyte reaction– and phytohemagglutinin M-induced lymphocyte reactivity were not due to cytotoxicity of the endometrial tissue culture supernatants, because the viability of lymphocytes cocultured with endometrial supernatants was usually >90%. The possibility that the inhibitory effects were caused by a complex reaction between endometrial supernatants and phytohemagglutinin M, thus making it unavailable for cell transformation, is not likely because the lymphocyte response could not be enhanced by increasing concentrations of phytohemagglutinin M with the constant dose of endometrial supernatants.

The immunologic characteristics of endometrial secretion are of considerable interest because sperm mi-

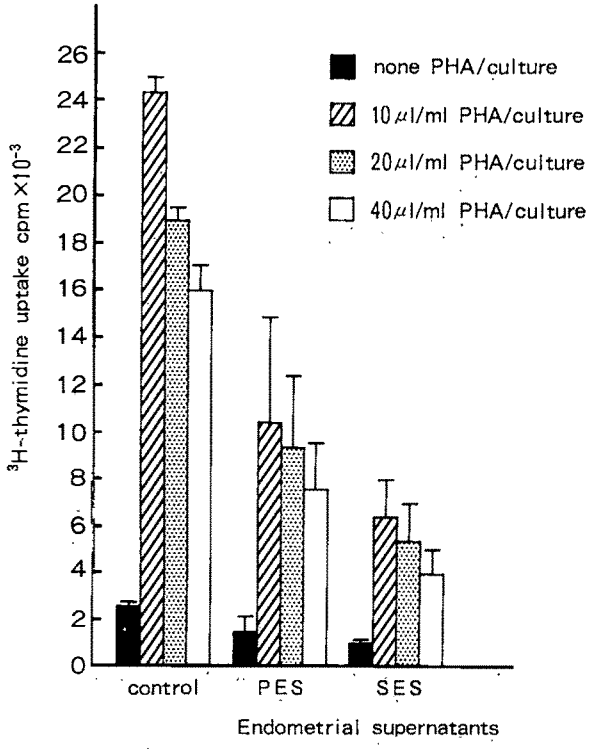


Fig. 5. Effect of various phytohemagglutinin *(PHA)* concentrations on lymphocyte stimulation (control) and on the suppression of lymphocyte activation by constant dose of secretory phase *(SES)* or proliferative phase *(PES)* endometrial supernatants. Results are expressed as counts per minute. Each value is the mean ± SD of three replications.

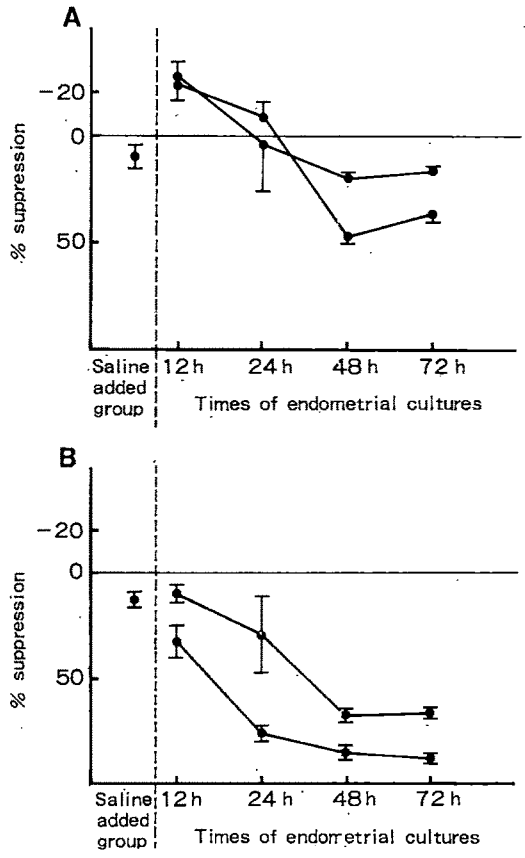


Fig. 6. Suppression of mixed lymphocyte reaction **(A)** and phytohemagglutinin-induced lymphocyte responsiveness **(B)** in relation to the times of endometrial culture. Results are presented as percent suppression in comparison with control cultures (peripheral blood lymphocytes plus Trowell's T8 medium plus allogeneic cells or phytohemagglutinin but without supernatant). Each value is the mean ± SD of three replications.

gration and blastocyst transport to the site of implantation occur in this fluid. Recently, many hormone-dependent substances were reported to be released from the endometrium,[4] but very little is known about their immunologic character. Tourville et al.[5] observed a strong IgG staining in the human endometrium, particularly during the secretory phase, and Menge and Lieberman[6] found that rabbit uterine fluid contained both IgG and IgA. Furthermore, lymphoid aggregates are found in the endometrial mucosa during the menstrual cycle.[7] Head and Seelig[8] showed that typical lymphatic vessels are present in the rat endometrium. Moreover, hormone-dependent large lymphoid cells, which suppress mitogen-induced lymphocyte reactivity, have been isolated from the human endometrium in the luteal phase.[9] These findings raise the possibility that the endometrium has a local immunoregulatory function.

During the last several years, some investigators have suggested that decidual tissue could fulfill a role in protecting the fetus from the maternal immune response by releasing some soluble immunosuppressive factor.[1,2] However, it is still unsettled whether the factor is already present in the endometrium before implantation or if some newly developed cells release it, as the possible bone marrow origins of some decidual cell precursors have been reported recently.[10] Our results indicate that the human nondecidualized endometrium contains a soluble suppressive factor involved in cell-mediated immunity and that the production of the factor increases as the menstrual cycle progresses.

The high frequency of early embryonic loss before implantation[11] and the low success rate of in vitro fertilization and embryo transfer in the human may be explained in part by the lack of some suppressive factor(s) in the endometrium. Elucidation of the mechanism of cellular and molecular events involved in the production or/and release of the factor(s) from human endometrium might shed light on a way to improve the treatment of infertility caused by implantation impediment.

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Magnesium and zinc status during the menstrual cycle

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The effect of menstrual cycle phases on the indexes of magnesium and zinc status was assessed in five normally menstruating women. Plasma concentrations of magnesium and zinc and the magnesium and zinc content of red blood cells and peripheral blood mononuclear cells were measured for 3 or 5 days per week during three menstrual cycles. The cycles were divided into four phases: menses, follicular, ovulatory, and luteal. Plasma magnesium concentrations were highest during menses and then gradually declinec, reaching the lowest point during the ovulatory phase. This was followed by a rise during the luteal phase. Plasma zinc concentrations were higher during menses and the follicular phase and then dropped during the ovulatory and luteal phases. Both the magnesium and zinc content of red blood cells and mononuclear cells remained constant over the course of the study and did not change significantly as a function of cycle phase. This study provides evidence that plasma magnesium and zinc concentrations fluctuate during the menstrual cycle in a phase-related fashion in normally menstruating women. Whether these changes are hormonally mediated or reflect metabolic changes will require further study. (AM J OBSTET GYNECOL 1987;157:964-8.)

Key words: Compartmentalization, follicular, luteal, menses, mononuclear cell, ovulation, red blood cell

The menstrual cycle is the result of complex interacting processes within the hypothalamus, the hypophysis, the ovaries, and the uterus.[1] Many changes occur within each of these different systems during the normal cycle, with each change dependent on a variety of sensitive regulatory mechanisms. Hormonal changes within each phase of the cycle have been well documented, but there is still uncertainty about specific mediators and regulators of the hormonal changes.

There is evidence that certain essential minerals may serve physiologic roles in regulating the release and/or biologic activity of hormones and neuropeptides involved in the menstrual cycle.[2-4] For example, zinc has been found to be involved in the regulation of progesterone[2] and prolactin activity.[3] In addition, recent data indicate that zinc ions may serve a critical role in endogenous opiate-receptor binding in the central nervous system.[4] Although no specific role has been assigned to magnesium in the regulation or maintenance of menstrual function, magnesium is involved in basal energy metabolism that changes over the course of the menstrual cycle.[5] Furthermore, it is believed that magnesium deficiency may be a possible cause for premenstrual tension syndrome.[6] This information suggests that distinct patterns in the distribution and/or metabolism of essential minerals might be observed during the menstrual cycle.

Patterns of change in the plasma concentrations of various minerals, such as calcium, phosphorous, magnesium, and zinc, across the menstrual cycle have been the subject of several research endeavors to delineate associations between hormonal changes and essential minerals.[7-10] However, the data are scarce, and whether there are clearly defined changes in the compartmentalization of these minerals has not been carefully studied. The purpose of this study was to evaluate changes in the plasma concentrations of magnesium and zinc as a function of menstrual cycle phase in healthy, normally menstruating women across three cycles. In addition, the magnesium and zinc content of red blood cells and peripheral blood mononuclear cells was measured to examine compartmental changes and yield more relevant information concerning the dynamics of magnesium and zinc metabolism during the menstrual cycle.

Material and methods

Informed consent was obtained from five healthy adult women, who ranged in age from 23 to 37 years, after they were instructed on the nature of the project. Three of the women were nulligravid, and two had two

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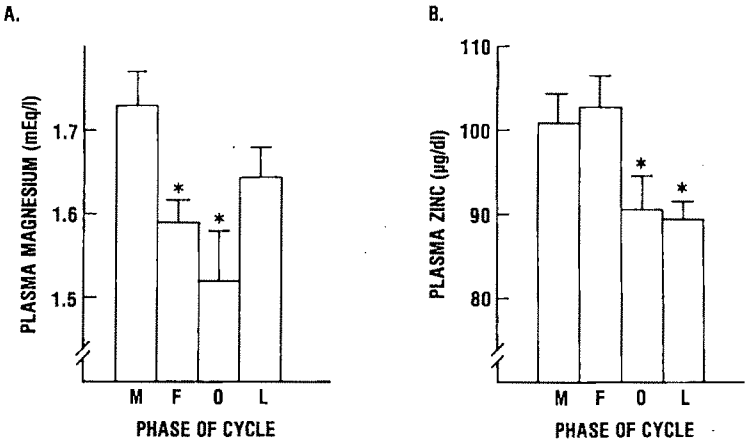


Fig. 1. Mean (±SEM) plasma concentrations of magnesium *(Panel A)* and zinc *(Panel B)* during menses *(M)*, the follicular *(F)*, ovulatory *(O)*, and luteal *(L)* phases of the menstrual cycle for five women. (*$p < 0.01$.)

and three previous term pregnancies, respectively. All had normal cycles, and none used any contraceptives or other medications. Furthermore, none suffered from premenstrual tension syndrome. The protocol had been approved by the Human Use Research Committee of the Uniformed Services University of the Health Sciences. Blood (25 ml) was drawn from an antecubital vein beginning on the first, second, or third day of the cycle (counting the onset of menses as day 1) at 8:00 AM after an overnight fast and collected 5 days a week until the onset of menses. The length of the menstrual cycle ranged from 24 to 31 days. By knowing the cycle length and basal body temperature, days within the menstrual cycle were classified into one of four phases: menses, follicular, ovulatory, and luteal. Values within each phase for each subject were grouped and averaged, and averages by phase were then calculated across subjects.

All blood samples were drawn into metal-free plastic syringes and transferred into three polypropylene tubes (Walter Sarstedt, Inc.; Princeton, N.J.): (1) no anticoagulant for whole blood measurements of magnesium and zinc concentrations and subsequent calculation of magnesium and zinc content of red blood cells, (2) ethylenediaminetetraacetic acid for complete blood cell counts, and (3) heparin for plasma concentrations of albumin, magnesium, and zinc and separation of mononuclear cells for magnesium and zinc content. Samples were processed immediately and any sample undergoing hemolysis was discarded.

Biochemical analyses. Red and white blood cell counts were determined on a Coulter counter Model ZM, and blood hemoglobin was measured with a Coulter Electronics hemoglobinometer. Hematocrit was determined in triplicate in microcapillary tubes centrifuged for 5 minutes at 12,000 rpm. Whole blood was hemolyzed by dilution with deionized water, vortexed, and then frozen for later analysis. Plasma from heparinized tubes was separated from whole blood by centrifugation and frozen for later analysis. Peripheral blood mononuclear cells were isolated from heparinized blood by the procedure of Elin and Hosseini.[11] In brief, this procedure involves the separation of mononuclear cells by centrifugation with an aqueous solution of sodium diatrizoate and Ficoll (Isolymph, Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, New York), harvesting, and counting the cells, which is followed by lysing the cells with deionized water and ultrasonication to release magnesium and zinc. Differential counts were performed on cytocentrifuge smears of harvested cells to document the percentage of mononuclear cells (>95). The magnesium and zinc concentrations in all samples were measured by atomic absorption spectrophotometry (Model 603, Perkin-Elmer, Eden Prairie, Minn.). Plasma albumin was measured on radial immunodiffusion plates with kits (Behring Diagnostics, La Jolla, Calif.). All tubes, containers, and reagents were carefully checked for contamination. Standard errors of measurement were less than 3% for all assays with the exception of the magnesium and zinc content of mononuclear cells, with standard errors of measurement of 6.8% and 7.5%, respectively.

Calculations. Magnesium and zinc content of red blood cells was expressed as nanograms per 10^6 cells and calculated as follows: (Whole blood concentration − plasma concentration × [(100 − HCT)/100])/RBC count where HCT is hematocrit and RBC is red blood cell. The magnesium and zinc content of mononuclear cells was also expressed as nanograms per 10^6 cells and calculated as lysate concentration/lysate cell count.

Statistical analyses. All data were analyzed by anal-

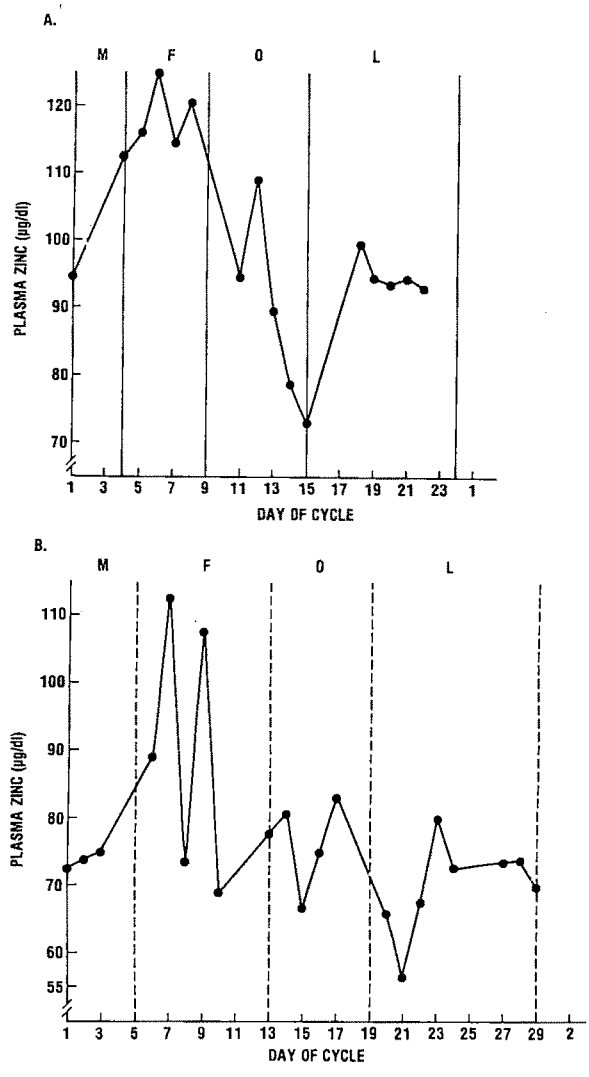


Fig. 2. Plasma zinc concentrations during the course of the menstrual cycle for two women with cycle lengths of 24 days *(Panel A)* and 29 days *(Panel B)*. (*M* = Menses; *F* = follicular; *O* = ovulatory; *L* = luteal.)

ysis of variance procedures to determine differences as a function of the four menstrual phases. When a significant effect was noted, Duncan's multiple range test was used. Standard correlation and regression procedures were used to assess interrelations between variables. The null hypothesis was rejected when $p < 0.05$.

Results

Mean plasma concentrations of magnesium and zinc for the subjects as a function of the four phases of the menstrual cycle are summarized in Fig. 1, *A* and *B*. There were significant differences in mean concentrations of both plasma magnesium and zinc across the four phases. Plasma magnesium concentrations varied in all subjects from day to day, but the values were highest during menses and then gradually dropped, reaching a low point during the ovulatory phase. During the luteal phase, plasma magnesium concentration began to rise.

Mean plasma concentration of zinc was highest during menses and the follicular phase and then dropped significantly during the ovulatory and luteal phases. Plasma zinc concentration tended to vary markedly during the follicular and ovulatory phases, and precipitous drops of 20 to 40 µg/dl were observed during these phases. The drops usually occurred within a 1-day period, between days 14 and 21, depending on cycle length. Patterns of change for two of the subjects with cycle lengths of 24 and 29 days are depicted in Fig. 2, *A* and B, respectively. As indicated, during the luteal phase, plasma zinc concentration was less variable than during the follicular or ovulatory phases. Although plasma albumin levels also varied considerably, with changes of up to 1.4 gm/dl noted across the cycle, the direction and magnitude of change did not always parallel that for plasma magnesium or zinc. However, the greatest declines were noted during the ovulatory phase.

The magnesium and zinc content of red blood cells and mononuclear cells exhibited considerably less day-to-day variation over the course of the menstrual cycle (Table I), and no significant patterns in these intracellular compartments were detected. When the magnesium and zinc content of red bloods was expressed relative to liters of blood hemoglobin, similar results were noted.

Comment

During the past several years, there has been increasing interest in the relationship between the metabolism of essential minerals and the regulation of hormone release and activity. Although the coordinated sequence of hormonal changes during the normal menstrual cycle is well characterized, whether similar or parallel changes occur in the distribution of selected minerals has not been clearly established. The results of this study provide preliminary evidence that there are specific phase-related patterns for plasma magnesium and zinc during the menstrual cycle despite marked day-to-day variation. In contrast, the content of magnesium and zinc in red blood cells and mononuclear cells remained relatively constant throughout the cycle.

Several studies have provided evidence for phase-related changes in blood constituents during the menstrual cycle,[7-10, 12] but various compartmental changes in magnesium and zinc have not been previously studied. The present study revealed that distinct changes occur in plasma magnesium during the menstrual cycle, with the lowest levels occurring during the ovulatory phase. This is consistent with the findings of Piffaretti[12] but not with those of Pitkin et al.[7] and M'Buyamba-

Table I. Mean content of magnesium and zinc in red blood cells and peripheral blood mononuclear cells averaged within phases during the menstrual cycle for normally menstruating women

	Menstrual cycle phases			
	Menses	*Follicular*	*Ovulatory*	*Luteal*
Red blood cells				
Magnesium	3.81 ± 0.14*	3.63 ± 0.14	3.87 ± 0.20	3.99 ± 0.15
Zinc	1.10 ± 0.04	1.10 ± 0.05	1.09 ± 0.04	1.18 ± 0.04
Mononuclear cells				
Magnesium	120.8 ± 8.7*	124.3 ± 6.4	116.0 ± 6.2	123.2 ± 4.7
Zinc	8.8 ± 1.2	7.7 ± 0.7	8.2 ± 0.7	8.1 ± 0.5

*Mean ± SEM; ng/10^6 cells.

Kabangu et al.[10] These investigators reported no significant patterns in plasma magnesium across the menstrual cycle. However, careful inspection of the data of Pitkin et al. reveals a midcycle drop. Thus it appears that there is a phase-specific pattern for plasma magnesium.

Although changes in albumin level may affect plasma magnesium concentration since albumin is reported to bind 25% of plasma magnesium,[13] the observed changes in plasma magnesium were independent of albumin levels. Thus other interpretations are sought. One possibility is that changes in plasma magnesium concentration are related to changes in basal metabolic rate in that both occur during midcycle.[5] Alternatively, the decline may be due to increases in parathyroid hormone or aldosterone, both of which increase before ovulation[7, 10] and induce decreases in plasma magnesium concentration.[14] However, the mechanism or signal responsible for the drop remains to be determined.

Although there are reports that the magnesium content of red blood cells is reduced in women with premenstrual tension syndrome, to the best of our knowledge no studies have examined patterns of change in the magnesium content of red blood cells or mononuclear cells during the menstrual cycle. The findings in the present study of no significant change in the magnesium content of either red blood cells or mononuclear cells provide evidence that the metabolism and distribution of magnesium in these tissues are probably independent of cycle phase in women with normal menstrual cycles. The data do not, however, rule out the possibility that a disturbance or abnormality in menstrual function might alter magnesium homeostasis. Conversely, whether a disruption in the metabolism or redistribution of magnesium contributes to the severity and/or onset of premenstrual tension syndrome remains to be determined.

The role of zinc in the reproductive function of women remains obscure, although several theories have been proposed. A relationship between zinc and progesterone binding[2] and zinc and prolactin release[3] has been proposed. Furthermore, there is evidence to suggest that zinc is an important modulator of opiate-receptor binding,[4] and endogenous opioid peptides participate in the control of gonadotropin secretion.[1] Thus there are several mechanisms whereby zinc might serve to regulate events in the menstrual cycle.

Patterns of change in plasma concentration of zinc have been previously reported,[9, 10] with peak values observed at ovulation. However, inspection of earlier data revealed minimal day-to-day variation. The results of this study also provide evidence for distinct patterns in plasma zinc concentration, but we found marked day-to-day variability. The pattern observed in this study was characterized by high mean plasma concentrations of zinc during menses and the follicular phase, followed by a decline to significantly lower levels during the ovulatory and luteal phases. Sharp rises and falls were noted during the late follicular and ovulatory phases, whereas during the luteal phase, plasma levels were remarkably stable.

Reasons for the observed changes in plasma zinc during the menstrual cycle are uncertain, but one might postulate that the sharp changes in plasma zinc may be related to hormonal changes or changes in the distribution of specific carrier proteins. Although albumin is the predominant carrier, binding 70% to 85% of zinc in plasma,[15] the differences observed in the present study could not be explained by variations in plasma albumin. Thus other explanations must be offered. For example, marked variations in plasma zinc concentration may be required to regulate the activity or binding of selected hormones.[2, 3] The precipitous decline during the ovulatory phase and the maintenance of low concentrations during the luteal phase may also be related to estrogen levels.[15] Alternatively, recent studies have provided evidence that plasma interleukin-1, which is associated with a reduction in plasma zinc,[15] is elevated during the luteal phase.[16] Thus interleukin-1 may be a regulator of zinc during the menstrual cycle. Finally, the low concentration of plasma zinc observed in this study during the luteal phase complements the results of Hag-

enfeldt et al.,[17] who reported higher levels of endometrial zinc during the luteal phase compared with the follicular phase. Zinc may be preferentially removed from plasma and taken up by endometrial tissue during the luteal phase to regulate progesterone and estradiol binding to tissue receptors. However, further studies will be required to address these particular issues.

No previous studies have explored potential interactions between intracellular zinc content and phases of the menstrual cycle. As was observed with intracellular magnesium, there were no particular patterns of change in the zinc content of red blood cells or mononuclear cells. There was day-to-day variation, but no distinct patterns as a function of cycle phase emerged. Because the intracellular zinc content of these specialized cells is not regulated by reproductive hormones, these tissues may provide a better reflection of actual zinc status.

In summary, the results of the present study provided evidence that the magnesium and zinc content of red blood cells and mononuclear cells does not change over the course of the menstrual cycle. In contrast, changes in plasma magnesium and zinc concentrations were noted. For plasma magnesium there was a gradual decline from menses to the ovulatory phase, followed by a rise during the luteal phase. Phase-related patterns in plasma zinc concentration were characterized by higher concentrations during menses and the follicular phase, followed by a decline during the ovulatory and luteal phases. Significant day-to-day variation was noted for both cations. Whether these changes are strictly an epiphenomenon resulting from hormonal changes or whether there is a deliberate redistribution of magnesium and zinc from plasma to other tissues wherein they serve regulatory functions is unknown. Future investigations will be required to answer these questions.

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Partial hydatidiform moles: Deoxyribonucleic acid content and course

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Partial moles are either diploid (46 chromosomes) or triploid (69 chromosomes). In 35 cases ploidy was measured by flow cytometry for nuclear deoxyribonucleic acid quantitation, indicating approximate chromosome number. Six of the 35 were triploid (17%) and the balance was diploid (83%). No complications occurred in the triploid group, while five of 25 (20%) diploid cases with evaluable follow-up had nonfatal sequelae. Complications included persistent mole treated by recurettage and four cases requiring chemotherapy (human chorionic gonadotropin titer plateau, clinical metastasis, overt choriocarcinoma, and placental site trophoblastic tumor). A combined morphologic and genetic classification of partial moles is recommended for identification and risk assessment. Deoxyribonucleic acid cell cycle fractions of the diploid partial moles may be helpful in the identification of patients at high risk for complications. A proliferation index >3.6 separated the cases with sequelae from most of the uncomplicated cases (sensitivity 100%, specificity 95%). (Am J Obstet Gynecol 1987;157:969-73.)

Key words: Partial mole, deoxyribonucleic acid content, chromosomal analysis

Makino et al.[1] in 1964 were the first to associate triploidy (69 chromosomes) with a placental mole of distinctive "transitional" structure. In 1976 two groups defined two molar entities as complete and partial. Vassilakos et al.[2] indicated that a slight majority of partial moles was diploid (46 chromosomes) and free of sequelae, whether triploid or not. Conversely, Stone and Bagshawe[3] reported that five of 189 partial moles (classified by morphologic criteria) required therapy for human chorionic gonadotropin titer persistence or clinical metastases. Since then there has been disagreement regarding the classification of partial moles (with morphologic criteria alone or an emphasis on cytogenetics) and the incidence of postmolar complications. Szulman and Surti[4] recognized partial moles typically as triploid and noted a low rate of sequelae in their series (two of 49). Lawler et al.[5] encountered only triploid partial moles, with no sequelae (15 cases, eight successfully karyotyped). Ohama et al.[6] reported 47 triploid cases and 9 diploid, with no complications. Series of partial moles diagnosed solely by morphologic criteria[7-9] show a higher complication rate (six of 83 total cases required treatment). Cases reported by Teng and Ballon[10] indicated two of three nontriploid partial mole cases required treatment, and an invasive mole was reported after a triploid partial mole by Gaber et al.[11]

Two pertinent questions arise: How frequent are diploid partial moles, and is there a risk difference between the two types of partial mole? Partial moles from our hospital were reviewed, their deoxyribonucleic acid (DNA) content was measured in tissue retrieved from paraffin blocks, and the clinical course of the patients was assessed. DNA quantitation was performed by flow cytometry, which allows approximate correlation with chromosome numbers. The sensitivity of the technique is sufficient to distinguish diploidy from triploidy, although differences of a few small chromosomes cannot be resolved.

Material and methods

Of the thirty-five cases of partial mole, all were retrieved from our surgical pathology files since 1981, except for one 1976 case previously karyotyped as triploid. Specimens were from curettage procedures, except for one in which hysterectomy was the primary treatment and one that involved salpingectomy for ectopic tubal pregnancy. Histopathologic review excluded both complete hydatidiform mole and hydropic abortion. The criteria of Szulman and Surti,[12] including fetal presence, placental membranes, villous vasculature, and partial villous involvement (normal and edematous villi), served to exclude a diagnosis of complete mole; criteria of trophoblastic hyperplasia (greater than normal for gestational age) and partial villous involvement eliminated hydropic abortion. In some instances the

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Table I. Partial mole cases

Patient No.	*Clinical*	*Weeks of gestation*	*Morphology*		*hCG (mIU/ml serum)*
			Vesicle (mm)	*TR*	
Triploid					
1	MAB	11	1.2	1+	
2	Mole	16	20	1	165K
3	Mole	17	15	2	1500K
4	MAB	9	1.2	1	
5	Mole	20	10	1	1.4
6	MAB	9	6	1	13.8
Diploid with complications					
7	Mole	9	4	2	597K
8	Mole	9	3.5	2	664K
9	IAB	10	1.5	1	20K
10	SAB	?	7	2	317 ↑
11	Mole	?	4	2	↑
Diploid without complications					
12	MAB	12	3	1	23
13	SAB	7	2	3	13
14	IAB	12	3	1	1.7
15*	Mole	14	4	4	344K
16	SAB	10	1	2	
17	IAB	10	1.5	3	
18	SAB	12	2	1	
19	IAB	10	1.5	1	2.6
20	SAB	11	3	1	199
21	IAB	12	2	1	100
22	MAB	16	4	1	118
23	SAB	8	1.5	2	39.9
24	Mole	12	6	3	729K
25	IAB	8	1.2	2	144
26	Tubal	8	1.5	3	66.3
27	SAB	10	1.3	1	
28	MAB	8	2	2	
29	MAB	10	1.8	2	
30	MAB	8	2	1	
31	MAB	18	5	2	37K
32	Mole	8	5	4	210K
33	Mole	11	5	4	970K
34	SAB	13	2	2	
35	SAB	?	3	3	

TR = Degree of trophoblastic hyperplasia (1 = slight, 4 = severe); hCG = human chorionic gonadotropin; PI = proliferation index; MAB = missed abortion; IAB = incomplete abortion; SAB = spontaneous abortion; Tubal = ectopic pregnancy; NE = diploid but not evaluable for cell cycle components; ↑ = rising titer; K = thousand.

*Hysterectomy without curettage.

clinical features of the mole further distinguished partial moles from abortions. Selected first trimester elective abortions were used as controls for the identification of trophoblastic hyperplasia. Medical records were reviewed for clinical presentation, gestational age, serum human chorionic gonadotropin titers, and follow-up.

The size of villous vesicles was recorded from gross specimen descriptions if available, or was measured from tissue sections. The degree of trophoblastic hyperplasia was recorded on a scale of 1 to 4 by selecting and grading the most intense hyperplasia prevalent in a low-power microscopic field (4.5 mm).

For DNA quantitation, representative samples were retrieved from paraffin blocks and prepared for flow cytometry according to the method of Schutte et al.[13] We introduced further disaggregation modifications that provided enhancement of DNA histograms. Briefly, the method involves manual slicing of thick sections from paraffin blocks with a scalpel to include chorionic villi with trophoblasts and maternal tissue for internal control, with sampling guided by a hematoxylin-eosin–stained section. Dewaxing in xylene and rehydration are standard. Our method included mincing slices with crossed scalpels followed by a novel enzymatic disaggregation. The sample was incubated at 37° C for 36 to 48 hours in 0.2% trypsin and 0.1% ethylenediaminetetracetic acid in phosphate-buffered saline solution A with 200 U/ml collagenase IV. Subsequent gentle syringing with 18- to 26-gauge needles,

DNA				
GO-1	S	G2-M	PI	Follow-up (mo)
74	18	8	0.9	108
83	12	5	0.3	1
89	7	14	2.2	15
63	17	20	6.4	1
88	4	8	0.7	6
68	16	16	3.8	5
64	17	19	5.6	14
47	35	19	7.7	16
	NE			3
65	9	26	10.4	31
33	56	11	3.7	36
76	11	13	2.2	24
71	15	14	2.8	10
77	20	3	0.1	24
30	25	45	67.5	36
80	6	14	2.5	0
80	7	13	2.1	21
64	32	4	0.3	8
81	17	2	0.1	16
57	20	22	8.5	15
59	28	13	2.9	3
59	38	3	0.2	12
70	12	8	0.9	9
62	24	14	3.1	12
77	17	6	0.5	11
64	21	15	3.5	2
74	22	4	0.2	3
69	27	4	0.2	7
67	29	5	0.4	9
	NE			5
95	0	5	0.3	4
77	19	4	0.2	3
74	25	1	0.0	1
	NE			?
51	12	37	26.8	?

filtration through a 30 μm nylon mesh, and washing provided enhanced harvests of cell suspensions. Staining was performed for 30 minutes at room temperature with ribonuclease (to eliminate ribonucleic acid), 1% NP40 (detergent), and propidium iodide (a DNA stain). With these added disaggregation methods, DNA histograms with coefficients of variation (sharpness of histogram peaks) <5% were considered good (EPICS V argon laser flow cytometer). Usually 10^4 nuclei were measured for DNA content in each case.

In addition to maternal tissue for an internal DNA quantitation control, an external control of paraffin-retrieved normal secretory endometrium was used for standardization of the histograms. The major objective was to classify partial moles as diploid or triploid. Cell cycle components were calculated from the histograms by PARA 1 analysis, a mathematic program for estimating cell proliferation characteristics. In Fig. 1 the histogram on the right has a normal diploid pattern; the 2n peak is the GO-1 diploid cell cycle fraction (nonreplicating chromosomes in modal range 46), the S fraction is the synthetic phase of chromosomes replicating DNA, and the 4n peak is the G2-M tetraploid fraction of fully replicated chromosomes before or at mitosis. The percentages are an imperfect representation of trophoblastic proliferation because GO-1 maternal and villous stromal cells were included, but sample parity allows rough comparison of S and G2-M fractions. Clinical, morphologic, and DNA quantitation data are listed in Table I. Five nonmolar abortions were used for trophoblast proliferation controls.

Table II. Proliferation index in diploid partial moles

Index	Complications	No complications
>3.6	4	1
<3.6	0	18
	4	19

Nonevaluable cases included Nos. 9, 30, and 34 (unanalyzable cell cycle fractions), 16 and 35 (lack of follow-up), and 15 (initial hysterectomy).

Results

Of the 35 cases, six were triploid (17%) and 29 were diploid (83%). Two cases (Nos. 1 and 3) of triploidy were confirmed by prior karyotyping. Only two cases in the diploid group were karyotyped. Case 8 was 46,XX; since parental karyotypes were not examined, the origin of the genome could not be evaluated (normal fertilization or androgenetic). Case 28 was trisomic 47,XX,+20; aneuploidy was too small to be detected by flow cytometry.

No complications occurred in the triploid group. Excluding four cases (Nos. 15, 16, 34, and 35) with nonevaluable follow-up, five of 25 (20%) diploid cases had sequelae. The sequelae were human chorionic gonadotropin titer plateau (case 7), plateau with clinical lung metastases (case 8), persistent mole (case 9), morphologically proved choriocarcinoma (case 10), and an aggressive placental site trophoblastic tumor (case 11). All five required chemotherapy, except case 9, which underwent recurettage. No fatalities have occurred. The human chorionic gonadotropin titers were negative at the designated follow-up in the nonsequelae cases (except case 2, which was lost to follow-up; Table I).

Abortion controls included a nonmolar case with DNA content in the triploid range, but with trophoblastic atrophy. Other controls were either nonevaluable for DNA content due to hypocellularity or were diploid with low proliferative characteristics.

Except for the proliferation index, no significant rela-

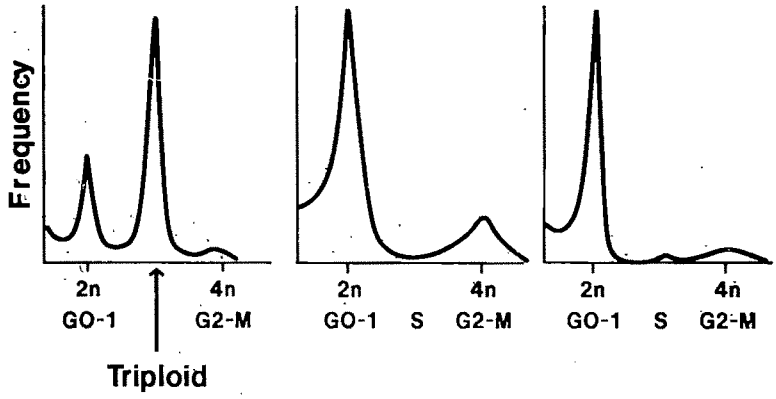


Fig. 1. Examples of DNA histograms. The y axis represents the number of nuclei measured; the x axis is fluorescence intensity representing DNA quantity in nuclei; 2n is within the diploid range of chromosomes (GO-1 cell cycle fraction); 4n is tetraploid range (G2-M fraction); and synthetic S fraction occurs at low levels in between. The first case (No. 1) is triploid *(arrow)*; the 2n and 4n peaks are internal control standards. The middle histogram is case 15, with a diploid pattern and high proliferation index (67.5). The last case (No. 12) is also diploid, with a low proliferation index (2.2).

Table III. Partial mole follow-up series

		Morphologic diagnosis		*Cytogenetic*			
				Triploid		*Nontriploid*	
Series	*No. of cases*	*Treated*	*None*	*Treated*	*None*	*Treated*	*None*
Vassilakos et al.[2]	56		7		22		27
Stone and Bagshawe[3]	194	5*	189				
Szulman and Surti[4]†	49		6	2	41		
Lawler et al.[5]	15		7		8		
Ohama et al.[6]	56				47		9
Goldstein and Berkowitz[7]	23	1	22				
Czernobilsky et al.[8]	25	1	24				
Wong and Ma[9]	35	4*	31				
	453	11	286	2	118	0	36

*Includes two cases with "metastasis."
†Of 86 cases, 49 were followed up.

tionships were present among the factors listed in Table I. However, as trends, the diploid cases with sequelae differed from triploid and diploid without sequelae in greater frequency of clinical mole presentation, <10 weeks' gestational age, trophoblastic hyperplasia ≥2+, human chorionic gonadotropin titer >20,000 mIU/ml serum, GO-1 ≤65%, and S phase ≥14%.

Because the complication cases had high G2-M cell cycle fractions, a proliferation index (PI) was calculated as: PI = $(G2\text{-}M)^2/GO\text{-}1$ (Tables I and II). All four evaluable complication cases and only one of 19 uncomplicated diploid cases ($p < 0.001$) had a proliferation index >3.6 (100% sensitivity and 95% specificity). The predictive value of this index was 80% positive and 100% negative. Fig. 1 includes diploid histograms with high and low proliferation indices.

Comment

Ascertainment of a partial mole has been a problem. Series of partial moles with follow-up information have either emphasized triploidy or were based on morphologic classification without cytogenetics (Table III). Cases classified cytogenetically[2, 4-6] included two of 156 (1.3%) requiring treatment, while in the cases classified morphologically[2-5, 7-9] 11 of 297 (3.7%) were treated for sequelae. Our combined DNA and morphologic approach should help to clarify questions of incidence and risk subsets of partial mole.

The true incidence of partial mole can be determined not only by distinguishing partial mole from complete mole, but partial mole from hydropic abortion. Only 10 of our cases were clinically moles, and nine had gross vesicles ≥5mm. Microscopic evaluation is necessary to

identify cases with microvesiculation. Perhaps more critical in partial mole recognition is differentiation from hydropic abortion. Our criteria stress trophoblastic hyperplasia for dates and partial villous involvement. To insist on a level of vesicle size would not allow recognition of early stages of molar development. Regardless of diagnosis of mole, all abortions with trophoblastic hyperplasia in our hospital are followed by human chorionic gonadotropin titer analysis. Careful scrutiny of "abortions" will yield cases of early partial mole. This raises speculation that some of the estimated 25% abortion antecedents for choriocarcinoma are unrecognized early moles (partial or complete).

Because complete moles and some partial moles are 46,XX, karyotypes alone may not be discriminatory. The androgenetic origin of complete moles is characteristic, although other fertilization mechanisms occasionally occur and may lead to confusion with partial mole cytogenetic patterns.[14] Morphologic criteria for classification of the two types of mole are well established; in partial mole there is evidence of fetal presence (gestational sac membranes and fetal vasculature with nucleated erythrocytes) and partial involvement (normal and vesicular villi). Although the two moles appear distinctive in origin, theoretically partial mole in advanced developmental stages could lose distinguishing features and simulate complete mole.

Our results more firmly establish that partial moles consist of triploid and diploid subsets, as others have suggested.[2, 15] Triploid partial moles are at low risk for sequelae, while diploid partial moles are at moderate risk. Our incidence of 20% sequelae is greater than the 15% accepted for complete moles, but is probably influenced by an ascertainment bias of cases 10 and 11. In both we encountered the sequelae before studying the antecedent mole. If these two cases are dismissed, the complication rate in the diploid group is three of 23 (13%). Further studies are needed to validate the risk level.

DNA quantitation of moles by flow cytometry is an easy, rapid method applicable to retrospective studies. It can also be used with fresh tissue as an alternative to cytogenetics. If morphologic features are the basis of partial mole diagnosis, DNA ploidy can serve to separate mole subsets without bias. A combination of chromosomal (or DNA) and morphologic evaluation is required for accurate assessment of biologic potential in molar disease.

A trophoblastic proliferation index >3.6 was arbitrarily selected as a characteristic of diploid partial moles with sequelae. This value was exceeded in only one of 19 uncomplicated diploid cases and two of six triploid cases. The role of the index as a risk factor requires further study.

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Correlation between chlamydia infection and clinical evaluation, vaginal wet smear, and cervical swab test in female adolescents

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Vaginal wet smear, endocervical swab test, gynecologic examination, and history were investigated in 148 healthy female adolescents. Cultures for *Chlamydia trachomatis* were obtained from all patients. The overall prevalence was 19/148 (13%). No significant correlation was found between chlamydia infection and any of the parameters investigated in the wet smear. Results of swab test or history showed no discrimination between young women with cultures positive for *C. trachomatis* and those with cultures negative for *C. trachomatis*. Chlamydia was found in 32% of patients when the gynecologic examination showed signs of infection and in 10% when no signs of infection were seen ($p < 0.025$). Thirteen of the 19 (70%) adolescents with chlamydia infections had no signs of infection. We conclude that neither swab test, vaginal wet smear, nor the history of the patient can give conclusive evidence of a chlamydial infection. Detection of *C. trachomatis* in this population can be done only by culture or other specific methods. (Am J Obstet Gynecol 1987;157:974-6.)

Key words: *Chlamydia trachomatis*, chlamydial infections, female adolescents

Chlamydia trachomatis is a major pathogen in genital infections that may cause serious complications such as salpingitis, infertility, and an increased risk for ectopic pregnancy.[1-6] Because of the high prevalence of *C. trachomatis* among teenage girls, often without symptoms,[2] tracing infected persons and performing epidemiologic studies are difficult unless large-scale screening procedures are used.

Brunham et al.[7] have described a simple cotton swab test for the presumptive diagnosis of chlamydial infection. The purpose of this study was to determine whether the cotton swab test can be used in a population of young women who have few symptoms and whether examination of vaginal wet smear is of value in making a presumptive diagnosis of chlamydial infection.

Material and methods

The study population consisted of 148 consecutive teenage girls who were attending an outpatient youth clinic. The mean age was 17.4 years (range from 14 to 21 years). All but five had had sexual intercourse. Two were pregnant.

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The clinic is served by two midwives with special training and experience in birth control and teenage counseling. A consulting gynecologist visits the clinic 2 hours a week. The clinic has approximately 3000 patients annually. About 90% of patients come for birth control information and the remainder for treatment of a variety of gynecologic problems, such as suspected infection, bleeding, abdominal pains, and sexual problems. Eighty percent of all teenage girls living in the area attend the clinic before the age of 20. Hormonal contraception is used by about 70%. None of the patients in the study population had an intrauterine contraceptive device.

A history was taken, with special reference to vaginal discharge during the preceding 3 to 4 weeks, malodor, metrorrhagia, itching, and dysuria. When one or more of these complaints were present, the patient was classified as having a suspected infection. In the absence of these the patients were classified as having no infection suspected.

The patients underwent a gynecologic examination by the midwife for erythema of the vaginal wall, erythema and/or edema, and fragility of any cervical ectopy, mucupurulent discharge from the cervix or vagina, and malodorous discharge. When one or more of these signs were present, the patient was classified as having signs of infection; if none were present the patient was classified as having no signs of infection.

The ectocervix was wiped clean with a large cotton swab. A white cotton swab was inserted in the cervical canal by the midwife and a gynecologist classified the color of the swab as white, yellow, or blood-stained.

Table I. Relation between chlamydia culture and leukocyte count, clue cells, and bacteria in vaginal wet mount preparation

	Chlamydia culture				*Total (N = 148)*
	Positive		*Negative*		
	n	%	*n*	%	
Leukocyte count (No. per high-power field)					
0-3	5	11	41	89	46
>3 but fewer than No. of epithelial cells	6	10	54	90	60
More than No. of epithelial cells	7	19*	30	81	37
Wet smear missing	1		4		5
Clue cells					
Present	10	18	47	82	57
Not present	8	9†	78	91	86
Wet smear missing	1		4		5
Bacteria					
Cocci-dominated	12	16	62	84	74
Rod-dominated	6	9‡	63	91	69
Wet smear missing	1		4		5

*$\chi^2 = 1.125$ (NS); $0.20 < p < 0.30$ (more leukocytes than epithelial cells against fewer leukocytes than epithelial cells).
†$\chi^2 = 1.433$ (NS); $0.20 < p < 0.30$.
‡$\chi^2 = 1.215$ (NS); $0.20 < p < 0.30$.

Table II. Correlation between *C. trachomatis* culture and leukocyte count in vaginal wet smear and objective signs of infection

Chlamydia culture	*Wet smear (leukocyte count)*	*Vaginal inspection*		*Wet smear missing*
		No signs of infection	*Signs of infection*	
Positive	0-3	5	0	1 SI
	>3	3	5	
Negative	0-3	39	2	3 NI, 1 SI
	>3	73	11	
Total		125	18	5

SI, Signs of infection; NI, no signs of infection.

With a second swab discharge was collected from the posterior fornix, placed on a slide in a drop of saline solution, and evaluated by the gynecologist by microscopic examination. The gynecologist did not have any clinical information or previous knowledge of the patient.

The following parameters were noted in the wet preparation.

Leukocyte count. There were more white blood cells than epithelial cells in all five different high-power fields (×400). There were more than three white blood cells per high-power field (average of five) but fewer white blood cells than epithelial cells. There were up to three white blood cells per high-power field (average of five) in a preparation with vaginal epithelial cells thus indicating adequate sampling technique.

Bacteria. Bacterial flora was dominated by large rod forms (Döderlein flora). Bacterial flora was dominated by cocci or by short rods.

Clue cells (epithelial cells coated with bacteria). Clue cells were noted as being present or not present. Culture specimens for *C. trachomatis* were taken from the endocervix with ear, nose, and throat swabs. They were transported to the laboratory in 2SP medium and on arrival were either cultured or frozen at −70° C until cultured (always within 1 week). Cultures were grown in monolayers of cycloheximide-treated McCoy cells in 24-well racks.[8] All cultures were read after 48 hours with a fluorescein isothiocyanate–conjugated monoclonal antibody for staining (MicroTrak Culture Confirmation, Syva Corp., Palo Alto, Calif.).

Statistical analysis. The χ^2 test with Yates' continuity correction was used for statistical comparison.

Results

Nineteen of the 148 (13%) patients in the study had positive cultures for *C. trachomatis*. Infection was suspected from the history in 17 patients. Two (12%) of

Table III. Sensitivity, specificity, and positive and negative predictive value of parameters studied compared with *C. trachomatis* culture

	Sensitivity (%)	*Specificity (%)*	*Predictive value*	
			Positive (%)	*Negative (%)*
Signs of infection at clinical examination	32	89	30	90
Swab test (yellow)	26	66	11	86
Infection suspected by history	11	88	12	87
Wet smear				
More leukocytes than epithelial cells	39	76	19	90
Clue cells present	56	62	18	91
Dominance of cocci or short rods	67	50	16	91

these had positive cultures and 17 (13%) of the 131 patients with no history of infection had positive cultures. Accordingly, 90% (17/19) of the patients with *C. trachomatis* had no subjective symptoms of infection. The clinical evaluation classified 19 as having suspected infection. Six (32%) of these had positive cultures for *C. trachomatis* compared with 13 of the remaining 129 (10%). This difference is significant ($p < 0.025$). When the swab test specimen was evaluated as white, 12% (7/58) had positive cultures. When the swab test specimen was yellow or blood-stained 11% (5/47) and 18% (7/39), respectively, were positive (not significant). The correlation between chlamydia cultures and the wet smear evaluation is shown in Table I. None of the differences observed were statistically significant. When the wet smear showed up to three leukocytes per high-power field and no signs of infection were seen by inspection, five of 44 (11%) had positive cultures (Table II). Trichomonas was not seen in any of the wet mount preparations.

Comment

Chlamydial infection is a common finding in sexually active women and is generally more prevalent in women under 20 years of age. The cultivation of *C. trachomatis* is a slow, technically demanding, and expensive procedure. Several reports have focused on the possibilities of making a presumptive diagnosis of chlamydial infection. Brunham et al.[7] found a good correlation between endocervical mucopus with a yellow stain on a cotton swab and the presence of chlamydia in a population undergoing testing for sexually transmitted diseases. Harrison et al.[9] found a good correlation between the absence of *C. trachomatis* and a lack of fragility and erythema of the cervix in college students.

Our findings show that neither the cotton swab test nor the absence of fragility/erythema could be used to exclude chlamydial infections in a patient population similar to ours. Only 30% of the women who had positive cultures had signs of infection on examination and the subjective symptoms gave no further help. We conclude that neither the swab test, the vaginal wet smear, nor the history of the patient can give conclusive evidence of a chlamydial infection. Detection of chlamydia in this population can be done only by culture or other specific methods (Table III).

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Evaluation of 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: A new tocolytic agent

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We attempted to characterize the ability of a new oxytocin derivative, 1-deamino[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin (ORF 22164), to antagonize the action of oxytocin in several in vitro and in vivo animal models of uterine hyperactivity. In these studies, the derivative was found to be a specific competitive inhibitor of oxytocin-induced contractions of pregnant guinea pig uterus in vitro. In addition, its intravenous administration induced a dose-dependent inhibition of oxytocin-induced uterine contractions in situ. Finally, like ritodrine, the drug induced a dose-dependent delay of ongoing labor in rats. These results suggest that 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin, unlike ritodrine, is a potent and specific antagonist of oxytocin-induced uterine contractions and thus may have potential clinical utility in the treatment of preterm labor. (AM J OBSTET GYNECOL 1987;157:977-82.)

Key words: Oxytocin, uterine hyperactivity, preterm labor

Much research has been conducted on preterm labor and labor-inhibiting drugs. However, the cause(s) of preterm labor is still not known, and the relative effectiveness and safety of various therapies are still unclear. Presently, the major treatment for preterm labor is the administration of selective β_2-adrenergic agonists that inhibit myometrial contractility by activation of receptor-mediated adenylcyclase. Unfortunately, treatment with β_2-adrenergic agonists has several liabilities, the primary one being the cardiovascular and metabolic side effects. In addition, although this therapy is very useful in the short-term treatment of preterm labor, its application in the long-term treatment of preterm labor is presently in question.[1, 2]

Oxytocin and vasopressin have been identified as possible causes of uterine hyperactivity in preterm labor.[3] A vasotocin derivative, 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin (deTVT; ORF 22164) has recently been reported to be a competitive antagonist of oxytocin[4] and may therefore be effective in inhibiting preterm labor. Our purpose was to examine the effect of the oxytocin antagonist deTVT in animal models for uterine contractility and preterm labor. Preliminary results of these studies have been published.[5]

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Material and methods

Animals. Virgin female Hartley guinea pigs (Dutchland Farms, Pennsylvania) and female Wistar rats (Charles River Breeding Laboratories, Boston, Massachusetts) were housed in climate-controlled light/dark (12-hour cycle) rooms and given food and water ad libitum. In those studies with pregnant guinea pigs or rats, females were housed with males and the presence of vaginal plugs was designated as day 1 of pregnancy. In those studies with nonpregnant guinea pigs, the first day of vaginal opening was designated as day 1 of estrus.

Uterine contractility studies

In vitro. Mature guinea pigs on day 20 of pregnancy were used in these studies because the uterus at this time is in a progestin-dominant state. A 10 mm uterine segment was removed and suspended on a weighted (1 gm) lever system in an organ bath containing 35 ml of a physiologic solution (Van Dyke-Hastings) containing low calcium (0.6 mg/ml) maintained at 37° C and continuously gassed with O_2:CO_2 (95%:5%). All uterine strips were allowed to equilibrate 60 minutes before the onset of experimentation. Isotonic contractions were measured with a Phipps and Bird ST-2 linear motion transducer coupled to a Beckman recorder. The physiograph recordings of myometrial concentrations were quantified by integrating the area under the curves and are expressed as arbitrary planimetric units per 5 minutes.

In situ. Uterine contractility was estimated by mea-

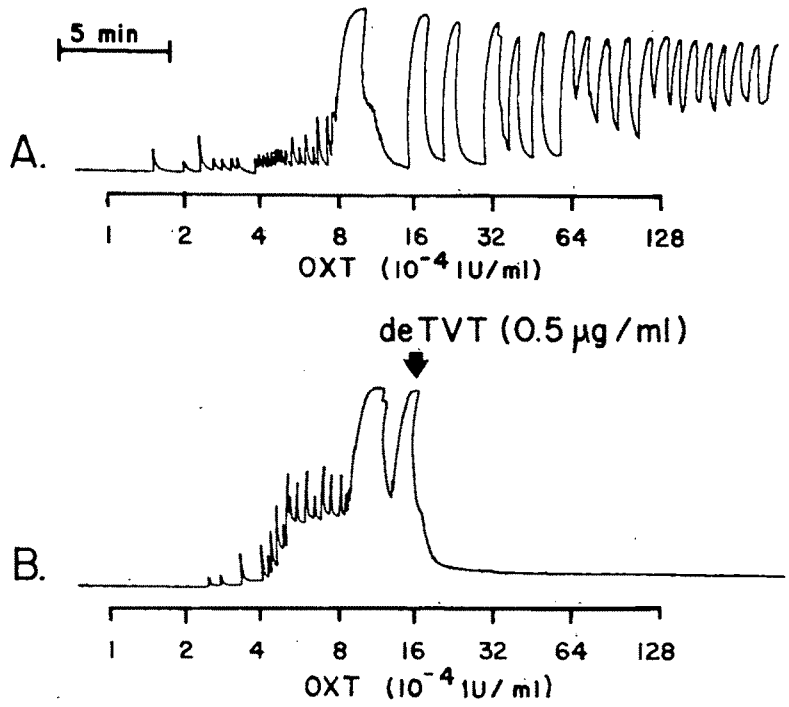


Fig. 1. The effect of deTVT on the oxytocin *(OXT)*-induced contractility of pregnant guinea pig uteri in vitro. Cumulative dose-response curves for oxytocin on myometrial strips were determined with **(B)** and without **(A)** the addition of deTVT (0.5 µg/ml).

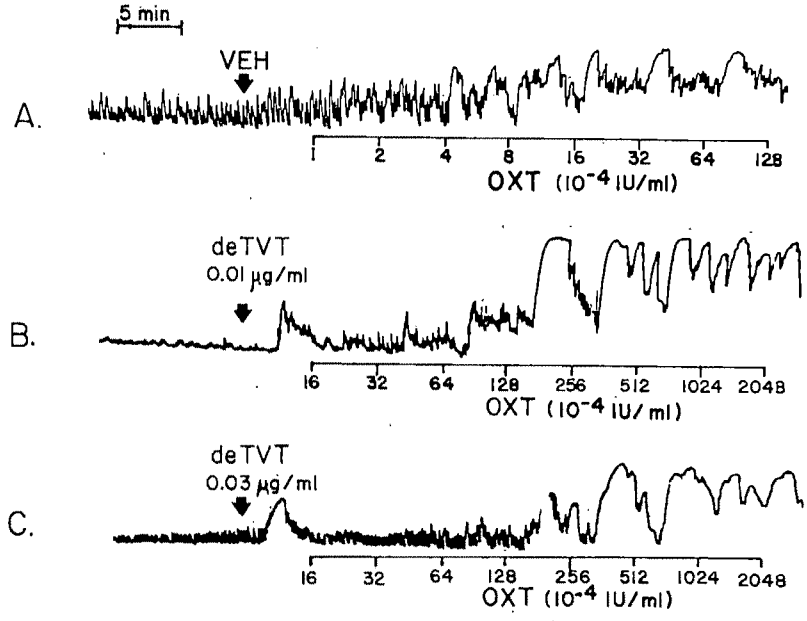


Fig. 2. Cumulative dose-response curve for oxytocin *(OXT)* on the contractility of guinea pig myometrial strips in vitro in the absence **(A)** or presence of deTVT, 0.01 **(B)** or 0.03 **(C)** µg/ml. *VEH*, Vehicle.

suring pressure changes in a cannulated perfused uterus in situ according to a previously published method.[6] Virgin female Hartley guinea pigs were anesthetized on day 1 of estrus with intraperitoneal pentobarbital sodium (35 mg/kg). Guinea pigs on day 1 of estrus (first day of vaginal opening) were used to ensure that the hormonal influences on the uteri were consistent. The external jugular vein was cannulated for drug

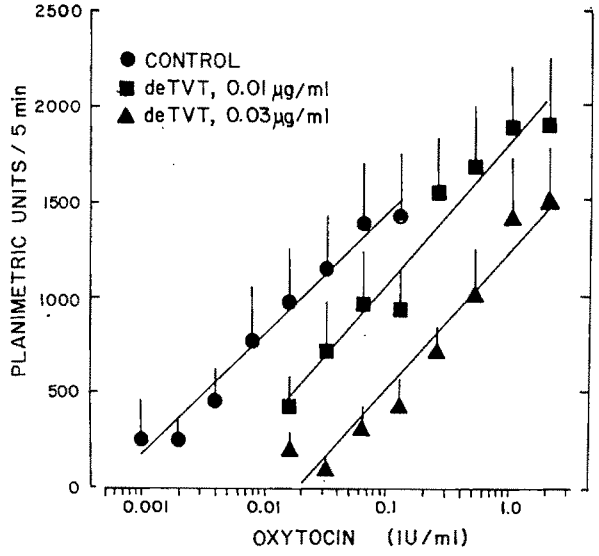


Fig. 3. Accumulated dose-response curves of oxytocin on the contractility of guinea pig myometrium in vitro with and without different concentrations of deTVT. Data are presented as the mean ± 1 SEM of five to seven determinations.

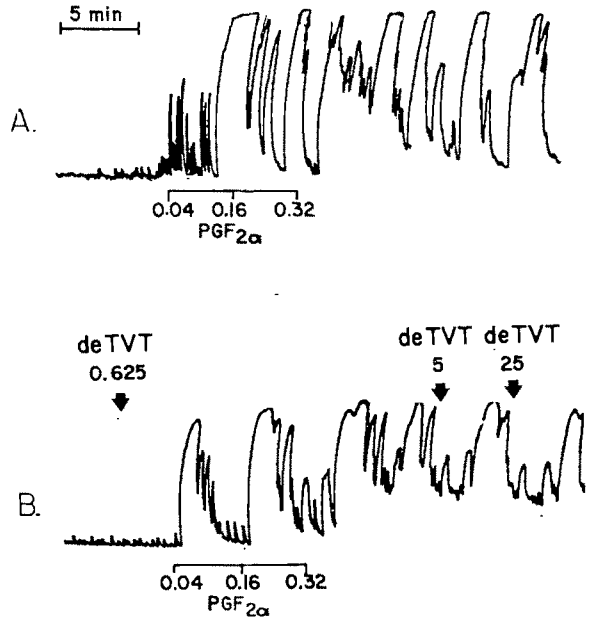


Fig. 4. The effect of deTVT on the prostaglandin *(PG)* $F_{2\alpha}$–induced contractility of pregnant guinea pig uteri in vitro. The effects of cumulative doses of prostaglandin $F_{2\alpha}$ (0.04, 0.16, and 0.32 μg/ml) were determined in the absence (**A**) and presence (**B**) of deTVT, 0.625, 5, and 25 μg/ml.

administration and the carotid artery was cannulated for the recording of arterial blood pressure. The uterus was cannulated at the uterotubal junction and a cannula was inserted through the cervix into the uterus and was ligated. The perfusate (distilled water at room temperature) was infused (0.5 ml/min) through the uterotubal junction cannula and was collected from the cervical cannula. Changes in uterine perfusion pressure were recorded with Beckman pressure transducers coupled to a Beckman Dynograph recorder. Responses in uterus tone were quantified in terms of millimeters of mercury pressure increases.

Preterm labor model, in vivo. The ability of drug treatments to delay the onset of labor was evaluated in a model that measures the spontaneous delivery time between the first and second rat pups in a term pregnancy.[7] Pregnant females were housed in individual plastic cages beginning on days 16 to 19 of pregnancy. On day 22 the females were observed continuously beginning at 8:30 AM. Immediately on delivery of the first pup, the female was administered the appropriate drug or vehicle treatment. The time of delivery of the first pup was recorded, and the female was returned to her cage and observed until delivery of the second pup. Data are expressed as time elapsed between the first and second deliveries.

Statistical analysis. Experimental data were initially analyzed with one-way analysis of variance. Between-group comparisons were made by the Student-Newman-Keuls test.[8] Differences with a probability of error <5% were considered significant.

Results

Uterine contractility studies

In vitro. An initial series of studies was undertaken to determine the effect of deTVT on oxytocin-induced contractions in isolated strips of pregnant guinea pig uterus. Oxytocin induced a dose-related increase in basal tone, amplitude, and frequency of individual contractions, with maximal effects obtained at 16×10^{-1} U/ml (Fig. 1). The administration of deTVT (0.5 μg/ml) completely abolished the oxytocin-induced contractions when dosed after maximal oxytocin-induced activity was reached. This antagonism was dose dependent and of a competitive nature (Figs. 2 and 3). When deTVT is administered before oxytocin, much higher concentrations of oxytocin are required to elicit the same degree of myometrial contractility. The cumulative results of these studies are summarized in Fig. 3. The dose-response curve for the effect of oxytocin on myometrial contractility is shifted to the right in a dose-dependent manner in the presence of increasing concentrations of the antagonist deTVT. This ability of deTVT to decrease the contractility of myometrial strips in vitro does not appear to be nonspecific action, because high doses of deTVT do not appear to affect prostaglandin $F_{2\alpha}$–induced uterine contractions (Fig. 4). These studies demonstrate that deTVT is a specific competitive antagonist of oxytocin-induced myometrial contractions in vitro.

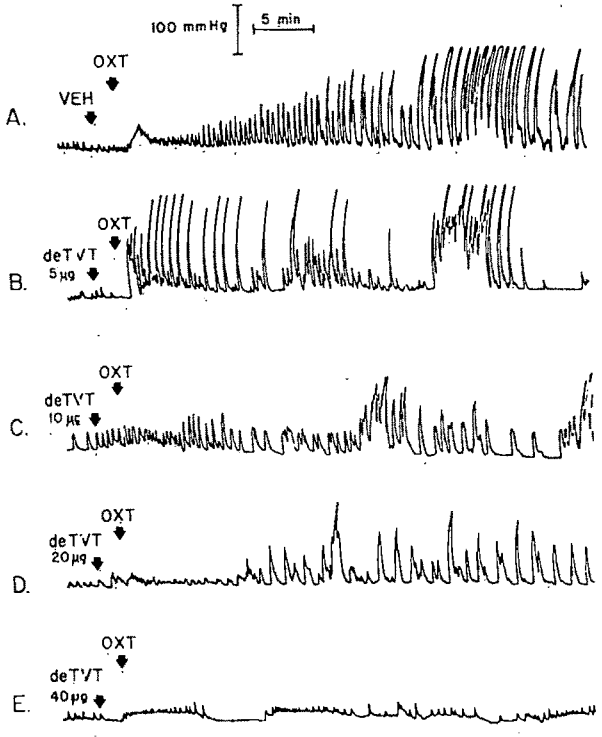


Fig. 5. The effect of deTVT on oxytocin *(OXT)*-induced uterine contractions in the guinea pig on day 1 of estrus in situ. All animals received an intravenous injection of oxytocin (0.4 IU) preceded by either an intravenous injection of deTVT or vehicle *(VEH)*. Uterine contractions were measured as changes in intrauterine pressure.

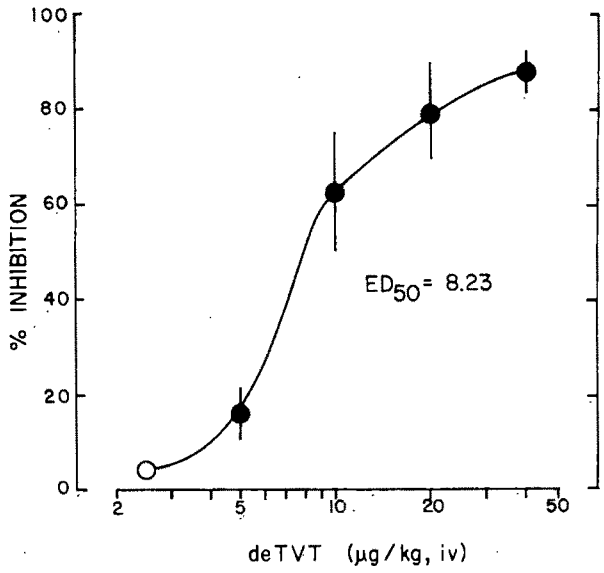


Fig. 6. Accumulated dose-response curves for the effect of deTVT on the inhibition of oxytocin-induced contractions in guinea pigs on day 1 of estrus in situ. Drug response was analyzed by integrating changes in intrauterine perfusion pressure over a 10-minute interval after dosing. Data are presented as the mean ± 1 SEM from five animals. The *solid circles* represent those values significantly different ($p < 0.05$) from control (0%).

In situ. The next series of experiments was undertaken to examine the ability of deTVT to block oxytocin-induced contractions in an in situ model of uterine hyperactivity. In these studies, oxytocin-induced changes in uterine contractility were quantified by measuring changes in uterine perfusion pressure in the presence or absence of deTVT in an anesthetized guinea pig on day 1 of estrus. Representative recordings from these studies are shown in Fig. 5. Intravenous oxytocin (0.4 IU/kg) induced a sustained increase in the frequency and amplitude of uterine contractions, as indicated by an increase in the intrauterine perfusion pressure. This oxytocin-induced increase was observed for at least 45 minutes. The administration of deTVT before oxytocin injection resulted in a dose-dependent decrease in both the frequency and amplitude of the oxytocin-induced contractions, with the highest dose (40 μg/kg) completely blocking the action of oxytocin. Fig. 6 clearly demonstrates that in situ deTVT antagonized the oxytocin-induced contractions of guinea pig uterus in a dose-dependent manner. The median effective dose for deTVT on the oxytocin-induced contractions of guinea pig uterus in this model was 8.23 μg/kg. These results demonstrate that deTVT is an effective antagonist of oxytocin-induced uterine contractions in situ.

Preterm labor model, in vivo. The final series of experiments was undertaken to compare the ability of deTVT and ritodrine, a β_2-antagonist, to antagonize spontaneous uterine contractions in an in vivo model of preterm labor.[7] In this model, pregnant rats were observed on day 22 of pregnancy (full term). Upon delivery of their first pup, either deTVT, ritodrine, or the respective vehicle was administered and the time until the delivery of the second pup was measured. Because deTVT is a peptide with a relatively short half-life, it (or its vehicle) was administered in doses of 100, 250, and 500 μg/kg subcutaneously every 5 minutes for 1 hour after the birth of the first pup; the maximal cumulative doses received were 1.2, 3.0, and 6.0 mg/kg, respectively. Both deTVT and ritodrine significantly delayed parturition compared with their respective vehicle-injected control groups (Fig. 7). Also, deTVT induced a dose-dependent delay in parturition, with the highest dose (500 μg/kg) being equivalent to the dose of ritodrine examined. These data demonstrate that deTVT, like ritodrine, is effective in vivo in delaying the progress of parturition after initiation.

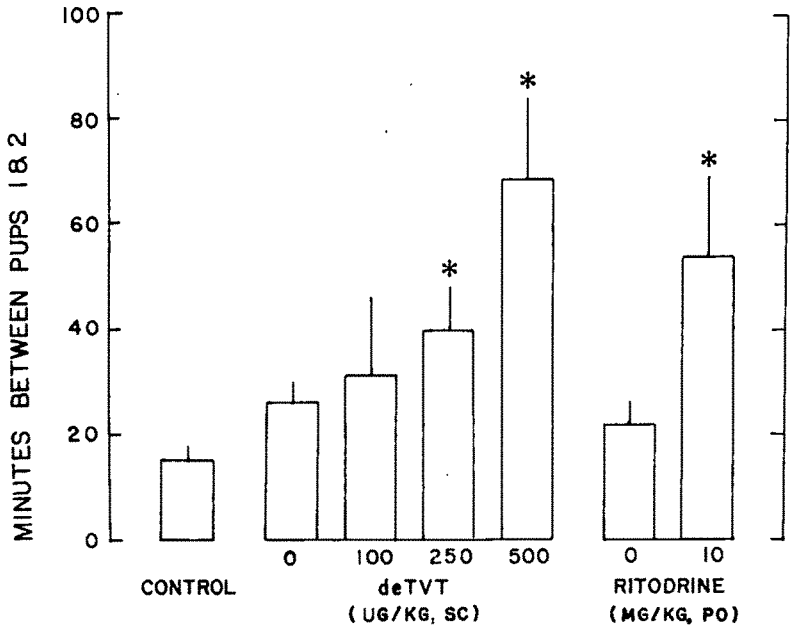


Fig. 7. Comparison of deTVT and ritodrine on the delay of parturition in rats. Each column represents the mean ± 1 SEM from five to six animals and is expressed as minutes elapsed between the deliveries of pup 1 and pup 2. *Significantly different ($p < 0.05$) from the respective vehicle-treated control.

Comment

Preterm birth is one of the major causes (75% to 80%) of perinatal morbidity and mortality. The social and economic consequences of preterm birth in terms of mental anguish and cost of caring for preterm infants and the associated lifelong infirmities are enormous. This is currently probably the most important problem to be addressed by modern obstetric research.[9]

At present, tocolytic treatment is not prophylactic, but commences after the onset of labor. The first line of treatment of preterm labor is the use of β-adrenergic agonists, but these agents are not the ideal therapy for preterm delivery. In fact, their use is associated with a high failure rate and a significant side effect liability.[9] The most common of these side effects is associated with β-agonist actions on the maternal cardiovascular system and metabolism.[10] Thus the development of specific new tocolytic agents without the side effect liability of the β-adrenergic agonists has an important clinical justification.

The present results demonstrate that deTVT is a competitive antagonist of oxytocin in vitro, as well as in vivo. In addition, these studies have demonstrated that deTVT is capable of delaying the progress of parturition after its onset in an in vivo model of preterm labor. These studies confirm previous results on the effect of deTVT on the oxytocin-induced contractions of rat and human uterine tissue in vitro.[4] Thus as an oxytocin antagonist, deTVT should be a highly specific agent with few side effects (since oxytocin receptors are found predominantly in uterus and mammary gland). Preliminary reports of clinical trials in which deTVT was effective in inhibiting uterine contractions during treatment of preterm labor support this contention.[11] In those clinical studies all patients reported a subjective relief of the undesired uterine activity and no side effects occurred. These pharmacologic properties make deTVT an ideal candidate for development as a new tocolytic agent.

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Stereotypic behavioral response of rat fetuses to acute hypoxia is altered by maternal alcohol consumption

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Transient compression of the umbilical cord has been implicated as a potential cause of unexplained brain damage and/or fetal death. We induced acute fetal hypoxia by compressing the umbilical cord of the fetal rat with a microvascular clamp. Direct observation of fetal behavior immediately after cord compression identified a three-phase behavioral response to acute hypoxia, consisting of an initial suppression in motor activity, a brief period of hyperactivity, and a secondary suppression of movement. All three phases occurred in <2 minutes from the onset of hypoxia. The stereotypic hypoxic response of rat fetuses may be adaptive as a means of removing mechanical sources of umbilical cord compression. Among fetuses exposed to alcohol in utero, the response to hypoxia was diminished in intensity and duration. Because of their altered hypoxic response, alcohol-exposed fetuses may therefore be at greater risk from transient cord compression in utero. (AM J OBSTET GYNECOL 1987;157:982-6.)

Key words: Fetal hypoxia, cord compression, fetal behavior, alcohol

Growth of the fetus and other changes that occur within the uterine environment increasingly confine the fetus during gestation and would seem to increase the chance of umbilical cord compression. Acute hypoxia resulting from compression of the umbilical cord in utero has been suggested as a significant source of brain damage and/or mortality among human fetuses.[1] There are a number of studies on the effects of hypoxia[2, 3] on fetal physiology. In most experiments, fetal hypoxia has been produced by manipulating oxygen availability to the mother, and fetal responses have been assessed by monitoring fetal heart rate or blood gases for an extended period. Little information exists on the immediate behavioral responses of fetuses to acute hypoxia, such as occurs after cord compression.

Our laboratory uses a technique that permits direct observation of rat fetuses during the last third of gestation. Briefly, pregnant rats receive a spinal anesthetic and are placed in a warm bath containing a physiologic solution. With the uterus externalized into this bath, fetuses may be delivered from the uterus, maintaining the placental-uterine attachment intact, and their behavior may be observed in detail.[4, 5] Thus the umbilical cord of rat fetuses can be compressed by application of a microvascular clamp, simulating a likely mechanism of umbilical occlusion in utero.

We report that acute hypoxia produced by clamping the cord is met by the rat fetus with a stereotypic behavioral response. The form and intensity of this response suggests that it may be adaptive in alleviating the source of cord compression. Rat fetuses exposed to alcohol in utero exhibit a diminished response to hypoxia, further suggesting that prenatal exposure to alcohol may place the fetus at greater risk from its own inadequate response to reverse transient hypoxia.

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Material and methods

Adult female Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts) were maintained under conditions of constant room temperature (22° C), regulated light cycle (12 hours light, 12 hours dark; lights on at 7 AM), and continuous access to food and water. Females were bred to Long-Evans males; this cross produces large litters of vigorous offspring ideally suited to prenatal study. Vaginal smears were taken over the period of breeding to identify the beginning of gestation (the first sperm-bearing smear was designated day 0). Animals were maintained and used in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23).

On day 20 of gestation, nine primiparous pregnant rats received a single oral intubation of a 20% w/v solution of ethanol in isotonic saline solution in a dosage of 4 gm ethanol per kilogram of female body weight. Ten nonintubated pregnant rats served as controls. (We have previously shown that unmanipulated fetuses are behaviorally equivalent to fetuses from mothers that receive a control intubation of isotonic saline solution.) Four hours elapsed between alcohol intubation and behavioral observation of fetuses. At this time the maternal blood alcohol content is 100 ± 20 gm/dl and a homogenate of fetal tissue shows an alcohol content of 78 ± 20 gm/dl.[6]

The methods used for preparation of the pregnant rat and subject fetuses are standard procedures in our laboratory and have been used extensively in studies of fetal behavior.[4, 5] With the rat under ether anesthesia, a chemomyelotomy was performed to chemically transect the rat's spinal cord between the first and second lumbar vertebrae, eliminating afferent stimuli from the lower body without the use of long-lasting general anesthesia. The duration of ether anesthesia was very brief (generally <60 seconds) and did not differ systematically between treatment groups. The prepared female was secured in a holding apparatus and her abdomen and hindlimbs were immersed in a bath of isotonic saline solution (Locke's solution) maintained at 37.5° C. The rat's uterus was externalized through a midline laparotomy and suspended just beneath the surface of the bath. A period of 15 to 20 minutes elapsed between the time of surgery and observation.

Two subject fetuses from each female were selected for behavioral observation. At the beginning of the observation period one subject fetus was delivered from the uterus and its amniotic sac into the bath, preserving the attachment of its placenta to the uterus. Immediately before the onset of observation, a microvascular clamp (RS-6470, Roboz Surgical Instrument Co., Washington, D.C.) was attached to the umbilical cord 5 to 10 mm from the fetus's abdomen. Attachment of the clamp applied 10 to 15 g of compression pressure, which was sufficient to effectively eliminate umbilical arterial and venous circulation during the observation period.

Beginning at the moment of attachment of the clamp, the fetus was observed for 2 minutes and each instance of its movement of the head, mouth, forelegs, rearlegs, or body trunk was recorded on a real-time event recorder. The sum of these individual movements provided a measure of total fetal activity. Because regional movements were defined independently, it was possible for them to occur at the same time. The incidence of such complex activity (the synchronous occurrence of two or more regional movements) was also examined. After observation of the first subject fetus, a second subject was delivered from the other uterine horn, the umbilical cord was clamped, and observation began. Only fetuses with good placental-uterine attachment were used as subjects. Comparison of observation records from different observers and reanalysis of videographic records have confirmed the high reliability of this observation protocol.[5, 7] Counts in the five regional categories and two derived categories (total and complex activity) were parsed into 5-second intervals over the 2-minute observation. Data from control and alcohol-exposed fetuses were compared in separate two factor repeated-measure analyses of variance. Experiments were carried out between 1 and 5 PM.

Results

Examination of the behavior of control fetuses from nonintubated mothers revealed a stereotypic response to transient hypoxia. Three distinct phases were identified in fetal behavior after occlusion of the umbilical cord (Fig. 1). In the first phase (approximately t_0-t_{30}), fetal movements decreased in frequency until they were almost completely suppressed. In a second phase (t_{30}-t_{75}) fetal activity rose sharply to a peak in excess of three times baseline rates of fetal movement. After the peak, a third phase was evident, as fetal movements again diminished in frequency until the end of the observation session (t_{120}). Fetuses exposed to alcohol exhibited less total activity than control fetuses ($F(1,36) = 13.1$, $p < 0.01$). Their activity peaked earlier and dissipated sooner than in controls, as indicated by the significant interaction between treatments and 5-second intervals ($F(23,828) = 2.0$, $p < 0.01$). Thus, although fetuses exposed to alcohol continued to show a response to hypoxia, it was diminished in magnitude and duration.

Examination of regional fetal movements indicated that only two categories—curl and head—differed when control and alcohol-exposed fetuses were compared (Fig. 1). In control fetuses, trunk curls occurred predominantly during phase II, coincident with the

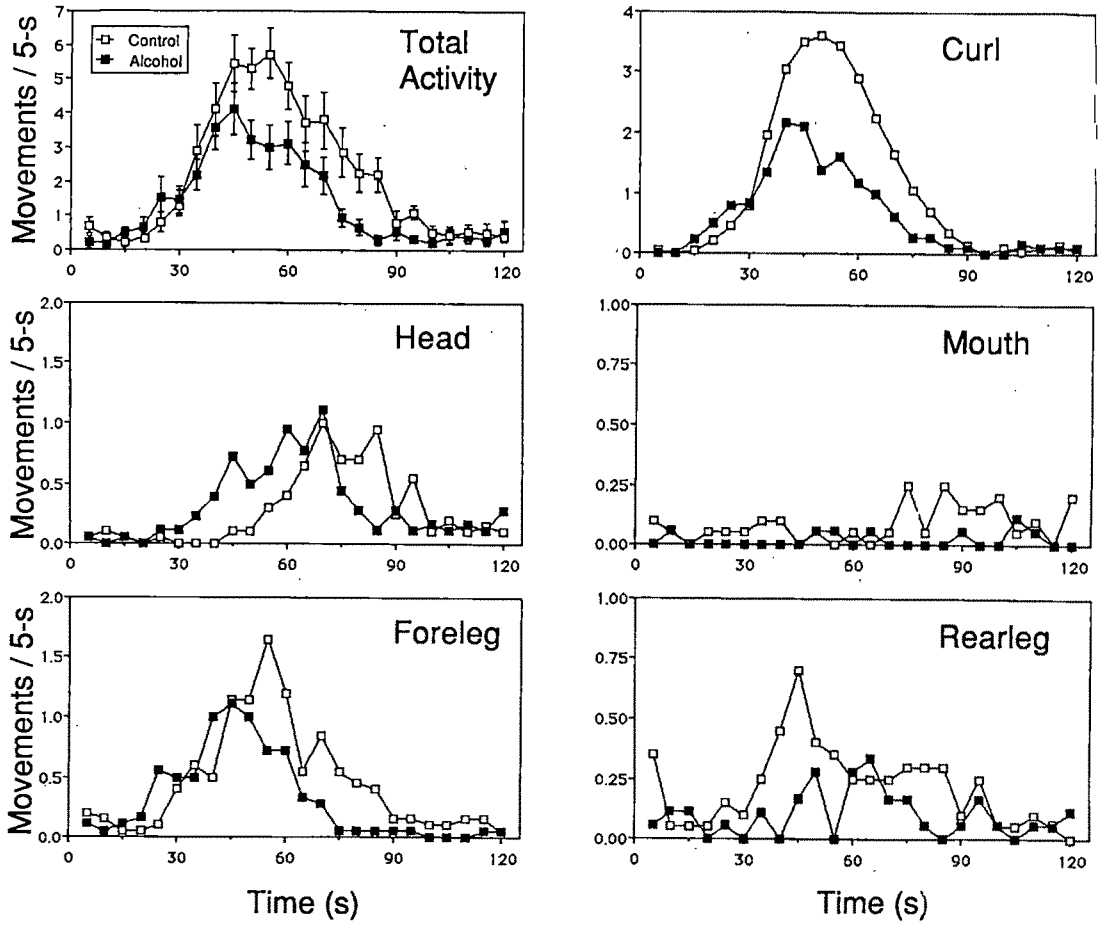


Fig. 1. Overall and regional activity of control and alcohol-exposed fetuses during 2-minute observations. The clamp was placed on the umbilical cord at t = 0. Points represent mean number of fetal movements per 5-second interval over the 2-minute observation. *Vertical lines* in the upper left figure depict the standard error of the mean. Unmanipulated 20-day-old rat fetuses move an average of 1.2 times every 5 seconds.[6]

sharp rise in overall activity. In fact, these vigorous lateral flexions of the body were the most common pattern of movement during the peak of activity. Head movements, in contrast, tended to occur during the secondary suppression of activity in phase III. Qualitatively, these head movements were also stereotypic, consisting of a rapid outward toss (cranial extension) of the head. Among alcohol-exposed fetuses these behavioral patterns continued to be associated with phases II and III, respectively. However, curls ($F(23,828) = 3.1$, $p < 0.01$) and head tosses ($F(23,828) = 2.2$, $p < 0.01$) occurred earlier in alcohol-exposed fetuses than in control fetuses. Three other categories of fetal movement (mouth, foreleg, rearleg) were uniformly suppressed after exposure to alcohol (all p values <0.01), but were not shifted in time relative to the onset of hypoxia.

In addition to simple movements, the incidence of complex activity (comprising two or more simultaneous movements) during hypoxic episodes was significantly suppressed by alcohol exposure ($F(1,36) = 8.2$, $p < 0.01$). Moreover, complex activity appeared to be much less common during hypoxia than among normal, well-oxygenated fetuses. We have previously described the emergence of complex activity during the last trimester of gestation in rat fetuses by comparing their occurrence with predictions from a model of random association of individual movements.[5] Briefly, if complex activities occur by accidental coincidence of simple movements, their frequency may be predicted as a relationship between the number of instances of fetal movement and the number of time intervals in which behavior is recorded. This relationship is expressed as:

$$f = B\left(\sum_{i=2}^{N} p^i\right)$$

where B is the number of intervals during an observation, N is the number of behavioral categories, p is

the probability of a fetal movement in an interval (estimated by the number of movements/B), and f is the predicted frequency of complex activity. This model of random association adequately accounts for the incidence of complex activity on days 16 and 17 of gestation, but by day 20 normal fetuses perform about 25% more complex activity than predicted.[5] We have interpreted these findings as evidence of increasing behavioral organization during the prenatal period. In contrast, under conditions of acute hypoxia, our control fetuses exhibited 68% fewer complex activities than predicted. This reduction in complex activity was not due to an overall suppression in activity; indeed, it occurred despite the exceedingly high rates of movement during phase II of the hypoxic response. Similarly, fetuses exposed to alcohol in utero performed 54% fewer complex activities than predicted during the period of hypoxia.

Comment

Clamping the umbilical cord appears to be a useful alternative to manipulating maternal oxygen availability to induce transient, acute hypoxia. Several minutes after the removal of the microvascular clamp at the end of the observation session, the rat fetus appears pink and well oxygenated, indicating that the clamp procedure has no lasting effect on umbilical circulation. Because the onset of umbilical clamping is well defined, it is possible to measure immediate and very brief responses to hypoxia that might otherwise go undetected. The reversible nature of umbilical clamping suggests that it may also be adaptable for use in the study of long-term consequences of transient hypoxia. As such, it may provide an animal model to explore the potential effects of transient compression of the umbilical cord during gestation.[1]

The period of time during which observations in this study were made, relative to the abrupt onset of hypoxia, is substantially shorter than has been used in previous studies of hypoxia.[2, 3] Indeed, the three phases of behavioral response to hypoxia are complete within 2 minutes of the placement of the umbilical clamp. The rapidity of the fetal response argues that transient episodes of hypoxia may be difficult to detect by traditional methods of examination and intermittent monitoring.

The brevity of the fetal hypoxic response does not, however, diminish its potential biologic significance. The striking reductions in synchronous activity suggest that hypoxia promotes a reorganization of fetal behavior. This interpretation is consistent with the view that the stereotypic lateral trunk curls and head tosses observed during acute hypoxia are specialized, reflexive movements that are not normally associated with the repertoire of spontaneous fetal behavior on day 20 of gestation. The stereotypy, intensity, and apparent reflexive nature of fetal behavior during acute hypoxia suggests that hyperactivity, and particularly lateral trunk curls and head tosses, are adaptive responses by the rat fetus to a sudden, deleterious change in its local environment.

If acute hypoxia may be induced in situ by pinching the umbilical cord against the wall of the uterus or the body of a sibling, as we believe likely, then vigorous outward-directed movements by the fetus may be useful in shifting body orientation or otherwise removing the source of the obstruction. Fetuses have been observed to change their body positions in utero and vigorous, whole-body movements have been implicated in effecting such changes.[8] The immediate motor response of fetuses to hypoxia is evidence of a kind of behavioral homeostasis ordinarily associated with adult animals: sudden detrimental changes in the intrauterine environment elicit behavioral responses from the organism that modify the uterine environment and restore favorable conditions.

Viewed in the context of adaptive behavior, the diminished activity of fetuses exposed to alcohol in utero assumes greater significance. Exposure to alcohol during gestation has been found to alter important features of the intrauterine environment, reducing amniotic fluid volume[6] and decreasing umbilical cord length.[9] A single exposure to alcohol is sufficient to cause transient constriction of the umbilical cord[10] and a marked suppression in fetal behavior. Fetuses exposed to alcohol are less active[6, 11] and less responsive to stimulation. The hypoxic response of rat fetuses exposed to alcohol is also diminished. Alcohol-exposed fetuses are less active overall and perform fewer lateral curls that subjectively appear less vigorous, and they perform these curls for a shorter period of time. They therefore appear to be less capable of responding adaptively to transient hypoxia and are less likely to have a mechanical impact on a source of umbilical cord compression. A single dose of alcohol administered to the mother is sufficient to suppress fetal activity for up to 4 hours.[6, 11] If the hypoxic response is impaired for a similarly extended period, then fetuses exposed to alcohol may be placed at significantly greater risk to transient hypoxia.

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Effects of norepinephrine on lung fluid flow rate in the chronically catheterized fetal lamb

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The influence of norepinephrine on lung fluid flow rate was studied in seven chronically catheterized fetal lambs. Lung fluid was collected through a catheter inserted into the fetal trachea. In six experiments, continuous saline solution infusion for 1 hour followed by equivolumnar norepinephrine (3.0 μg/min) for 1 hour via the fetal jugular vein was carried out. In three additional experiments (two fetuses), a 3.0 mg intravenous bolus of propranolol was administered before norepinephrine infusion. Saline solution infusion did not influence the lung fluid flow rate or fetal biophysical and biochemical parameters. Norepinephrine increased fetal blood pressure by 22% and decreased lung fluid flow by 45% from control values. Lung fluid flow rate recovered within 2 hours after the end of the norepinephrine infusion. Pretreatment with propranolol did not alter the results. In addition to previous reports that demonstrated β-sympathomimetic agonist–enhanced reabsorption of lung fluid from fetal alveolar epithelial cells, our present results suggest that a reduction in fetal lung fluid secretion may be implemented as well through stimulation of α-sympathomimetic receptor sites in the pulmonary vasculature. (Am J Obstet Gynecol 1987;157:986-90.)

Key words: Fetal tracheal fluid, fetal lung fluid, fetal lamb, norepinephrine, propranolol

The fetal trachea, bronchi, and alveoli are filled with liquid during the prenatal period. This fluid was believed to be an aspirate of amniotic fluid until Olver et al.[1] confirmed that the major portion of fetal lung fluid was secreted from pulmonary capillaries into alveoli. Secretion of the fluid progressively increases as gestational age advances in fetal sheep,[2] and a large part of the lung fluid formed in the fetal lungs spills into the amniotic cavity.[3]

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Recently, major interest has focused on the dynamics of fetal lung fluid production and the influence of catecholamines. Walters et al.[4] demonstrated that the secretion was inhibited at the level of epithelium by β-adrenergic receptor stimulation. There has been a common clinical observation of decreased amniotic fluid associated with the chronically distressed fetus or in the fetus with intrauterine growth retardation. In both conditions elevated catecholamine levels have been reported.[5]

The purpose of our study is to investigate the influence of exogenous norepinephrine on the fetal lung fluid dynamics in chronically instrumented fetal lambs.

Material and methods

Seven time-mated pregnant ewes (cross-bred Rambouillet-Columbia Suffolk, single gestation 127 to 132 days) were used. The pregnant ewe fasted 24 hours

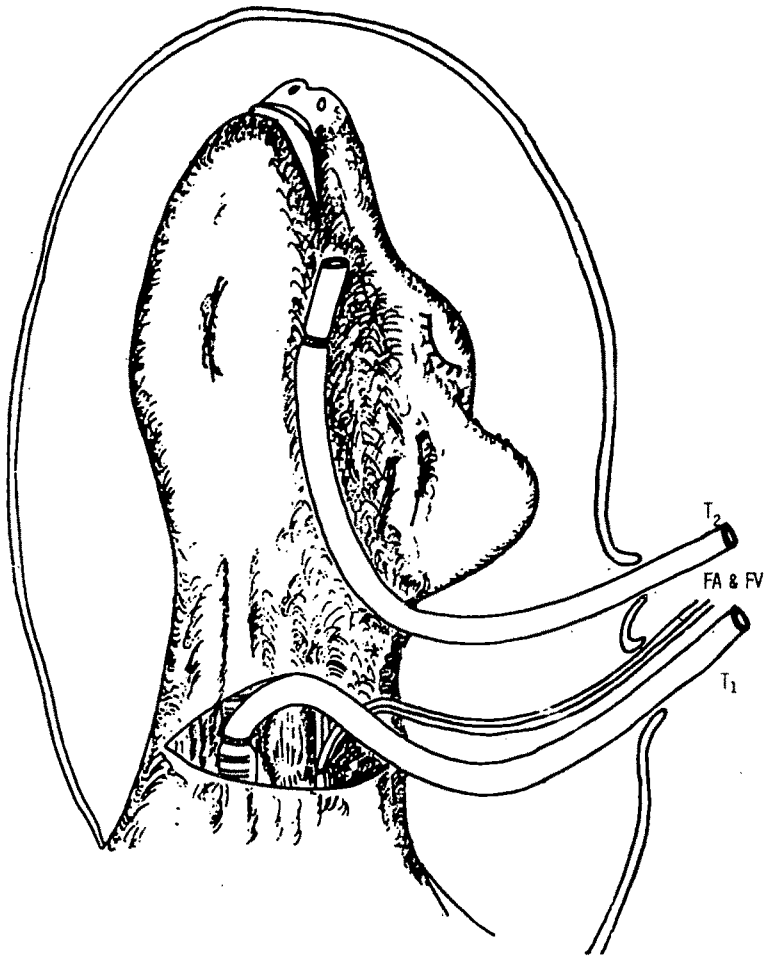


Fig. 1. A tracheal catheter (T_1) inserted in the fetal trachea and sutured securely for quantitative collection of fetal lung fluid. Another catheter (T_2) was connected to return lung fluid into the amniotic cavity while the experiments were not being performed. *FA*, Fetal artery catheter; *FV*, fetal vein catheter.

before surgery. The mother was anesthetized by intramuscular xylazine, 12 to 15 mg, and additional local anesthesia with 1% Xylocaine was given as needed during surgery. The uterus was exposed through a paramedian abdominal incision and the fetal head was delivered. Inspiration of air into the fetal lungs was prevented by covering the fetal head with a surgical glove filled with warm saline solution. Electrocardiogram electrodes were sutured under the fetal chest skin. The fetal carotid artery and jugular vein were catheterized. An open-end catheter was left inside the amniotic cavity to record urterine activity.

A catheter (4 mm inside diameter, 7 mm outside diameter, 100 cm in length) was inserted and sutured securely in the fetal trachea for quantitative collection of lung fluid (T_1, Fig. 1). A similar catheter was sutured on the fetal face with an open end located near the mouth (T_2, Fig. 1). The maternal femoral artery and vein were also catheterized. All catheters and elecrodes were exteriorized through the maternal flank.

The animals were allowed to recover in an air-conditioned stall where food and water were given ad libitum. During recovery, the two large catheters were connected to allow fluid produced in the fetal lungs to flow back into the amniotic cavity.

Experiments were performed on the fifth postoperative day or later. Before each experiment the catheter from the fetal trachea was connected with a sterile tubing to a sterilized polyvinyl collection bag. The tubing tip draining into the bag was adjusted in height to equal the resistance to fluid drainage into the amniotic cavity. Collection of fetal lung fluid was started 1 hour before the experiment to assure stable flow was achieved.

The protocol for the experiment consisted of the control period, saline solution infusion (0.1 ml/min), and norepinephrine infusion (3.0 μg/min = 0.1 ml/min), each of which lasted 1 hour. Postinfusion observation was maintained for 2 hours after the end of norepinephrine infusion (Fig. 2). Fetal arterial blood

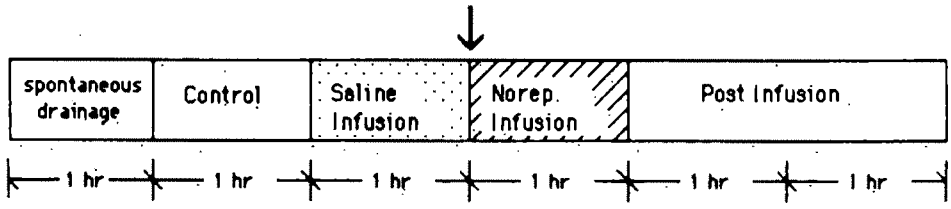


Fig. 2. Experimental protocol for quantitative collection of fetal lung fluid. Saline solution was infused at the rate of 0.1 ml/min; norepinephrine was infused at 3.0 μg/min in the same volume. Two fetuses (three experiments) received an intravenous bolus of propranolol, 3.0 mg, before norepinephrine infusion *(arrow)*.

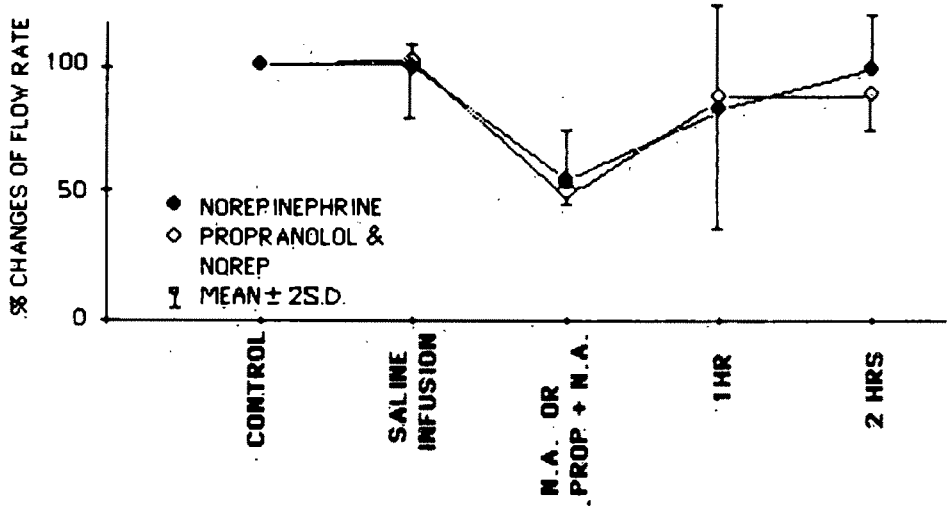


Fig. 3. Percent changes in fetal lung flow rate during saline solution infusion, norepinephrine with or without pretreatment with propranolol, and 1- and 2-hour recovery periods.

pH and gases and tracheal fluid flow rate were determined for each hourly collection period. Fetal heart rate and arterial pressure were continuously recorded on a multichannel recorder.

Six experiments were conducted in five fetuses following this protocol. An additional two animals (three experiments) received a bolus injection of propranolol (3.0 mg) before norepinephrine infusion. Electrolytes in the lung fluid obtained during the control and experimental periods were also analyzed.

Results

The mean (±SD) values of fetal arterial oxygen and carbon dioxide pressures and pH immediately before the experiments were 18.1 ± 1.6, 44.8 ± 1.4, and 7.37 ± 0.05 mm Hg, respectively. No appreciable changes occurred during the experiments. The average fetal heart rate was not consistently altered by norepinephrine infusion. The mean fetal arterial blood pressure exhibited an average increase of 22% from control.

Table I shows changes in the fetal lung fluid flow rate in five fetuses that received norepinephrine (six experiments) and two animals that received propranolol and norepinephrine (three experiments). The average lung fluid flow rate during the first observation period was 14.1 ml/hr (3.5 ml/kg/hr). Saline solution infusion did not significantly alter the flow rate. There was a significant decrease in the flow rate by 45.3% from control during norepinephrine infusion ($p < 0.001$, paired t test). The rate of fluid flow recovered within 2 hours after discontinuation of norepinephrine (Fig. 3).

Norepinephrine infusion after propranolol administration also decreased the fluid flow rate by 45.5% from control. This was not significantly different from the effect of norepinephrine alone ($p > 0.05$, Student's *t* test).

Table II shows electrolyte concentrations in the fetal lung fluid during control and norepinephrine infusion periods. No significant differences were observed.

Table I. Fetal tracheal fluid flow (ml/hr)

Fetus	*Control*	*Saline solution*	*Norepinephrine*	*Post infusion*	
				1 hr	*2 hr*
1	14.6 (100%)	13.0 (89.0%)	6.3 (43.2%)	—	—
2	17.0 (100%)	15.0 (88.2%)	8.0 (47.1%)	10.0 (58.8%)	18.0 (105.9%)
3	18.0 (100%)	18.0 (100.0%)	10.0 (55.6%)	12.0 (66.7%)	15.0 (83.3%)
4	13.0 (100%)	13.0 (100.0%)	7.0 (53.8%)	9.0 (69.2%)	12.0 (92.3%)
4	12.0 (100%)	14.0 (116.7%)	7.0 (58.3%)	13.0 (108.3%)	12.0 (100.0%)
5	10.0 (100%)	10.5 (105.0%)	7.0 (70.0%)	11.0 (110.0%)	11.8 (110.0%)
Mean	14.1 (100%)	13.9 (99.8%)	7.5 (54.7%)	11.0 (82.6%)	13.6 (98.3%)
±SD	3.0	2.5	1.3	1.6	2.7
Propranolol plus norepinephrine					
6	9.0 (100%)	9.0 (100.0%)	4.0 (44.4%)	6.0 (66.7%)	7.4 (82.5%)
7	15.2 (100%)	15.8 (103.9%)	7.8 (51.3%)	14.0 (92.1%)	13.0 (85.5%)
7	14.5 (100%)	14.6 (100.6%)	7.0 (48.3%)	15.0 (103.4%)	14.0 (96.6%)
Mean	12.9 (100%)	13.1 (101.5%)	6.3 (48.0%)	11.7 (87.4%)	34.4 (88.2%)
±SD	3.4	3.6	2.0	4.9	3.6

Comment

The average hourly production of fetal lung fluid we observed during the control period was 3.5 ml/kg in the fetal lambs at approximately 130 days' gestation. This result agrees well with previous reports of 2.5 and 4.0 ml/kg by Normand et al.,[6] 4.5 ml/kg/hr by Mescher et al.,[7] and 300 to 400 ml/day cited by Enhorning et al.[8]

The mechanisms responsible for producing such a large amount of fluid in the fetal lungs are still not clear. Olver and Strang[1] have suggested a mechanism by which water is moved from the interstitium into the potential air spaces by active transport of chloride ion in the pulmonary epithelium against the gradient of protein osmotic pressure between lung alveoli and interstitial fluid or fetal plasma. Enhorning et al.[8] reported that the lung weight of fetal rabbits was reduced by treatment with isoxsuprine, and that the phenomenon was due to a decrease in the pulmonary fluid. Long-term administration of terbutaline, a selective β_2-receptor stimulant, to pregnant rabbits has also been shown to decrease the fetal lung-body weight ratio and to enhance secretion of surface-active material into amniotic fluid.[9] A marked decrease in tracheal fluid flow and increase in pulmonary surfactant efflux after the infusion of epinephrine to fetal sheep were also reported.[10] Furthermore, Brown et al.[11] observed that this epinephrine effect in reducing the lung liquid secretion became more remarkable with advancing gestation of the fetus. Walters and Olver[4] demonstrated that isoproterenol and epinephrine inhibited secretion of fetal lung fluid, and that the inhibitory effect was blocked with propranolol. The authors concluded that there was direct β-adrenergic stimulation of a liquid secretion mechanism at the pulmonary epithelial level.

Table II. Electrolytes in fetal tracheal fluid (mEq/L)

	Sodium	*Potassium*	*Chloride*
Control	138 ± 2.0	5.9 ± 0.4	201 ± 8.0
Norepinephrine	137 ± 1.0	4.8 ± 0.3	200 ± 7.0

Data are mean ± SD.

In our study, norepinephrine infusion significantly decreased the lung fluid flow rate, and the response was not modified by pretreatment with propranolol. The mechanism of flow rate reduction, therefore, is presumed to differ from the β-adrenergic stimulation at the pulmonary epithelium.

An increase in fetal pulmonary vascular resistance as a possible mechanism is supported by the observations made by Assali et al.[12] and Cassin et al.,[13] who separately reported a rise in pulmonary arterial pressure during norepinephrine injection in fetal sheep. It is therefore speculated that norepinephrine infused in the fetal lamb reduces the lung fluid net flow through its α-adrenergic effects on the pulmonary vascular beds.

Bland et al.[14] suggested and Kitterman et al.[15] demonstrated that a reduction of lung fluid in the fetal lamb under physiologic conditions may begin before birth. Brown et al.[11] attributed this to an increased plasma concentration of epinephrine during early labor. Reduced lung fluid in infants delivered vaginally is a common clinical observation. Human infants delivered vaginally without complication have been shown to have elevated catecholamine levels in cord blood,[16] and even moderate hypoxia has been reported to increase catecholamine levels markedly in fetal sheep.[17] A decrease in lung fluid secretion associated with parturition

and/or fetal hypoxia may be a preparatory process to clear the potential air space for the neonatal period.

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Characteristics of the electromyogram recorded from the mesometrium of the pregnant ewe from 106 days' gestation to delivery: Similarities with and differences from the electromyogram obtained from the myometrium

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Electromyographic recordings from 12 pregnant sheep were analyzed in four gestational age groups (106 to 115, 116 to 125, 126 to 135, and 136 to 145 days) and by the day of delivery. Mesometrial electromyographic activity was present in all animals studied. Contracture activity was synchronous in both mesometrium and myometrium. The frequency distribution of electromyographic events of different duration in the mesometrium was qualitatively identical to that recorded from the myometrium. The frequency of events <180 seconds was greater in the myometrium, while no difference was observed in contracture frequency. Mean contracture duration in myometrium and mesometrium shortened as gestation progressed ($p < 0.01$), and was of significantly shorter duration on the day of delivery when compared with all gestational age intervals ($p < 0.05$). Except at 135 to 145 days gestation and at the day of delivery, myometrial contractures were of significantly longer duration than mesometrial contractures. The presence of the endometrium and fetal membranes in the pregnant ewe may stimulate the myometrium, and account for the difference observed. In vivo recording of mesometrial electromyographic activity may provide an opportunity to study the paracrine regulation of myometrial activity. (Am J Obstet Gynecol 1987;157:991-8.)

Key words: Electromyography, mesometrium, endometrium

Myometrial electromyographic activity has been recorded in several species, in both nonpregnant[1-5] and pregnant[6-10] animals. Much of this work has been conducted in the sheep, because it is possible to relate myometrial electromyographic activity to a broad data base of physiologic information. Reports describing electromyographic activity based on visual analysis of frequency and duration of electromyographic events, while of value, lack precision on event quantification and definition of events with differing durations. Recordings of intrauterine pressure have also been used to characterize myometrial activity.[6,8] Visual evaluation of intrauterine pressure traces from short-term recordings, in terms of frequency and/or duration, may lose information. It is also difficult to use simple visual analysis to obtain information on patterns of activity over longer periods in pregnancy. In addition, intrauterine pressure changes during contractures are small and difficult to distinguish from movement and other potential artifacts.[11] The information obtained from myometrial electromyographic recordings is different from that obtained with intrauterine pressure recordings. Electromyographic recording represents the activity of all the various input factors that lead to the depolarization of the myometrial cells. It hence reflects a set of physiologic features different from those of the intrauterine pressure, which also involves changes in stimulus-contraction coupling.

With the use of continuous recording and computerized analysis, we have reported the myometrial electromyographic characteristics in the pregnant sheep after surgery.[9] Our data acquisition system and computer software allow us to determine the frequency distribution of electromyographic events of different duration. We have recently reported the electromyographic activity of the mesometrium in the nonpregnant ovariectomized sheep.[5] Mesometrial muscle fibers form a single, unidirectional layer of muscle continuous with the external layer of the myometrium. In the non-

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Table I. Outcome of individual sheep preparations

Animal no.	Gestational age at surgery	No. of hours of recording studied				Gestational age at termination
		Group A	Group B	Group C	Group D	
116	140	—	—	—	—	142
142	127	—	—	—	48	142
190	117	—	96	168	72	138
204	119	—	72	240	48	139
207	119	—	96	240	240	151
219	122	—	—	—	144	145
221	105	48	240	144	48	148
222	105	48	—	—	—	120
223	105	48	240	240	—	142
224	105	48	240	144	—	146
237	117	—	—	—	120	150
238	117	—	—	—	48	143

Table II. Comparison of electromyographic events according to duration

Group	N	Events <1 min					Events >1 min and < 2 min				
		Myometrium		Mesometrium			Myometrium		Mesometrium		
		Mean	SD	Mean	SD	p	Mean	SD	Mean	SD	p
A	4	339	189	199	86	0.13	40.5	22.3	23.3	9.6	0.11
B	6	327	216	98	38	0.02	39.0	21.0	14.8	8.3	0.03
C	6	316	132	129	106	0.02	33.5	13.0	15.8	10.7	0.02
D	9	261	132	108	49	0.01	37.3	24.0	10.5	4.4	0.01

pregnant ewe, mesometrial electromyographic activity showed both similarities with and differences to myometrial activity. Contracture frequency was significantly greater in the mesometrium than in the myometrium both before and after estradiol administration to the nonpregnant ovariectomized ewe. Estradiol increased the short-term electromyographic events (<180 seconds) in the myometrium but not in the mesometrium. These differences may be attributed to paracrine regulation by the endometrium that underlies the myometrium but not the mesometrium.

Our aims were to record the electromyographic changes from electrodes placed on myometrium and mesometrium during the last third of gestation, to characterize the frequency distribution of electromyographic events of different duration, to compare mesometrial electromyographic activity with that recorded from the uterine horns, and to determine how the recordings at these two sites change throughout the last third of gestation and on the day of delivery.

Material and methods

Experimental animals. Twelve pregnant ewes of known gestational age were instrumented. Surgery was performed under halothane general anesthesia. Pairs of electromyographic electrodes were placed in myometrial and mesometrial tissue as previously described.[5, 6] In four of the ewes no further instrumentation was performed. Maternal and fetal vascular catheters were placed in the other eight ewes. The day of surgery was considered day 0. No analysis was performed until day 5 after surgery.

Recording of uterine electromyogram and data analysis. Uterine electromyographic activity was recorded and stored in a custom-built data acquisition system.[9] Event quantification was performed with a 2-hour running mean to set the threshold for distinguishing signal from noise. A 16-second period of activity below the threshold (the delimiter) was selected to determine when an event was completed. Recording of electromyographic activity was continuous and was analyzed in 24- and/or 1-hour blocks during the study. For frequency distribution analysis, electromyographic epochs were divided into 1-minute groups of burst duration lasting less than 1, 2, 3, 4, 5, 6, 7, 8, or 9 minutes. For further analysis, events were divided into two categories, <180 seconds (noncontracture activity) or >180 seconds (contracture activity). Mean contracture duration was calculated for each analysis block in each animal. The time for the noncontracture and contrac-

Outcome
Delivery recorded; live lamb in good condition
Sheep transferred to different protocol; fetus alive and well
Sheep transferred to different protocol; fetus alive and well
Sheep transferred to different protocol; fetus alive and well
Delivery recorded; live lamb
Delivery recorded; live lamb
Delivery not recorded; live lamb
Twin gestation
Delivery recorded; live lamb
Delivery recorded; live lamb
Delivery recorded; live lamb
Delivery recorded; live lamb

ture activities during which electrical activity was above threshold (total time active) during the analysis period was computed in each analysis block.

For convenience of analysis and to observe any gestational age–related trends, the study period was divided into five groups: four based on gestational age and also a group studied on the day of delivery. Data were available from four sheep from 106 to 115 days' gestational age (group A), six from 116 to 125 days' gestational age (group B), seven from 126 to 136 days' gestational age (group C), eight from 136 to 145 days' gestational age (group D), and seven on the day of delivery (delivery group).

Statistical analysis

Data were analyzed on the basis of the number of events per unit time, mean contracture duration (seconds), and total time active per 1-hour interval. Analysis on a 1-hour time base was considered necessary to avoid missing any rapid changes that may occur at the time of delivery. Data were available for at least 2 days in each animal included in any 10-day window (Table I). The total number of hours recorded from each animal are shown in Table I. Except on the day of delivery, only units of 24 hours of recording were analyzed, to avoid problems arising from circadian rhythms.[12]

The data from individual 24-hour intervals for each sheep were averaged to give one data point for each gestational age interval. The overall mean (±SD) was calculated from these data points. Means were compared between groups with the two-sample independent Student's t test. The Bonferroni correction was applied when multiple comparisons were performed. One-way analysis of variance was used for multiple mean comparisons. Statistical significance was set at the 0.05 level. Data throughout are presented as the mean ± SD.

Results

Characteristics of mesometrial electromyographic recordings. Mesometrial electromyographic activity was present in all animals studied. Fig. 1 shows the recording of mesometrial and myometrial electromyographic events and intrauterine pressure obtained from a pregnant sheep at 130 days' gestational age. Contracture-type activity was synchronous in all electromyographic leads as well as with the intrauterine pressure trace.

The frequency distribution for the number of electromyographic events of different duration in both myometrium and mesometrium at the four different gestational age intervals is shown in Fig. 2, *A* and *B*, and Fig. 2, *C* and *D*. The profile for the mesometrium is similar to that we have described for the myometrium in the pregnant sheep during the first 8 days after surgery, in that there are two populations of electromyographic events.[9] Noncontracture activity follows an exponential function and contracture activity follows a gaussian function. At all gestational age intervals (except 106 to 115 days), the number of shorter events (those <1 minute and those >1 but <2 minutes) in the myometrium was always greater than in the mesometrium ($p < 0.05$; Table II). In view of the clear separation of electromyographic events into two distinct groups, further analysis and comparison was based solely on two categories, noncontracture and contracture activity.

Characteristics and comparison of the two types of myometrial and mesometrial electromyographic events during the last third of gestation

Frequency of electromyographic events. Fig. 3 compares the frequency of noncontracture activity and contracture activity for the different intervals. Myometrial and mesometrial noncontracture and contracture frequency did not change significantly throughout the period of analysis. The frequency of events <180 seconds was greater in the myometrium than in the mesometrium. This difference was statistically significant at 116 to 125 and at 136 to 145 days' gestational age. Contracture frequency was not different at the two recording sites at any gestational age.

Mean contracture duration and total time active. Fig. 4 shows the mean contracture duration in both myometrium and mesometrium at the four different gestational age periods and on the day of delivery. Mean contracture duration shortened in both myometrium and mesometrium as gestation progressed ($p < 0.01$). Mean contracture duration was statistically shorter the day of delivery in both myometrium and mesometrium when compared with all other gestational age intervals ($p < 0.05$). Contractures were of significantly longer duration in the myometrium than in the mesometrium

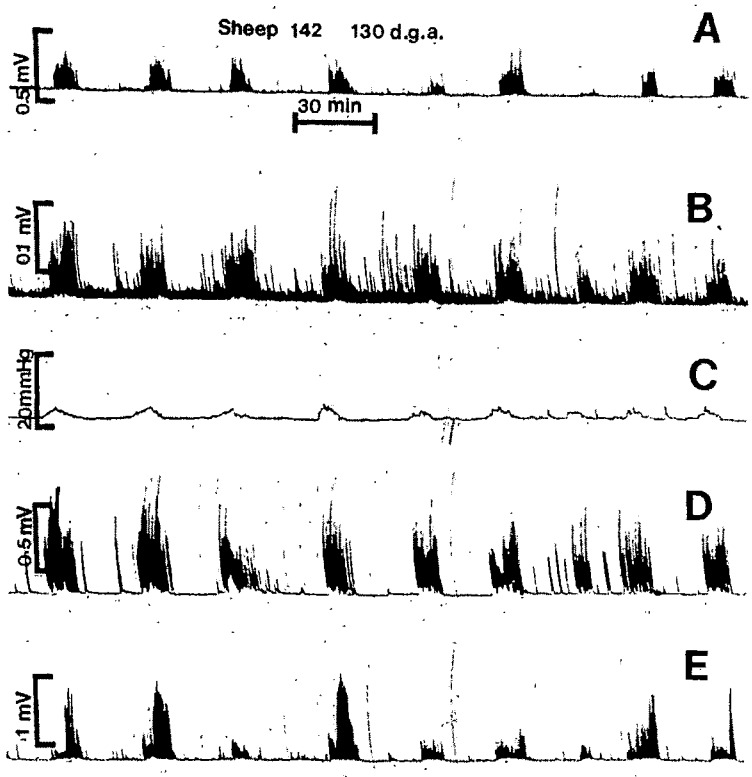


Fig. 1. Electromyogram recordings from a pregnant sheep at 130 days' gestation. **A,** Left mesometrium; **B,** myometrium of nonpregnant horn; **C,** amniotic pressure; **D,** myometrium of pregnant horn; **E,** right mesometrium.

at all gestational ages except 136 to 145 days and at delivery ($p < 0.05$).

Total time active (the sum of noncontracture and contracture activities) did not change during the last third of gestation in either myometrium or mesometrium. Although total time active was greater in the myometrium than the mesometrium at all intervals, it was statistically different only at 126 to 135 days' gestational age. In the mesometrium the total time active increased significantly on the day of delivery ($p < 0.05$) when compared with all other gestational age intervals (Fig. 5). Although total time active increased in the myometrium, this increase did not reach statistical significance.

Comment

Mesometrial electromyographic activity was present in all the pregnant ewes studied. Mesometrial activity was qualitatively identical to that recorded from the myometrium of the uterine horns. The frequency distribution of myometrial and mesometrial activity showed a profile identical to the one we have previously described for the myometrium of the pregnant sheep in the period immediately after surgery. Contracture electromyographic activity is generally, although not always, accompanied by an increase in intrauterine pressure. It is still not clear how to interpret noncontracture electromyographic activity. The noncontracture type of electrical activity is present throughout gestation, but only at delivery is it closely coupled with mechanical events. It is important to ask why the myocyte is able to depolarize and contract to produce the low-amplitude, long-lasting intrauterine pressure changes characteristic of contractures, but not the short, higher amplitude changes of contractions. It is possible that increased gap-junction density and/or permeability, such as occurs at the time of delivery, allows myocytes to contract synchronously, therefore developing more tension.[13-15] This possibility still does not explain the shorter duration of labor and delivery contractions. In addition, it is necessary to consider the effect of changes in the viscoelastic properties of the uterine connective tissue on the pattern of myometrial activity and the resultant effects of intrauterine pressure. We have previously shown that contracture-related electromyographic activity can be recorded simultaneously over most of the uterus.[16] Thus the low intrauterine pressure increment generated is not due

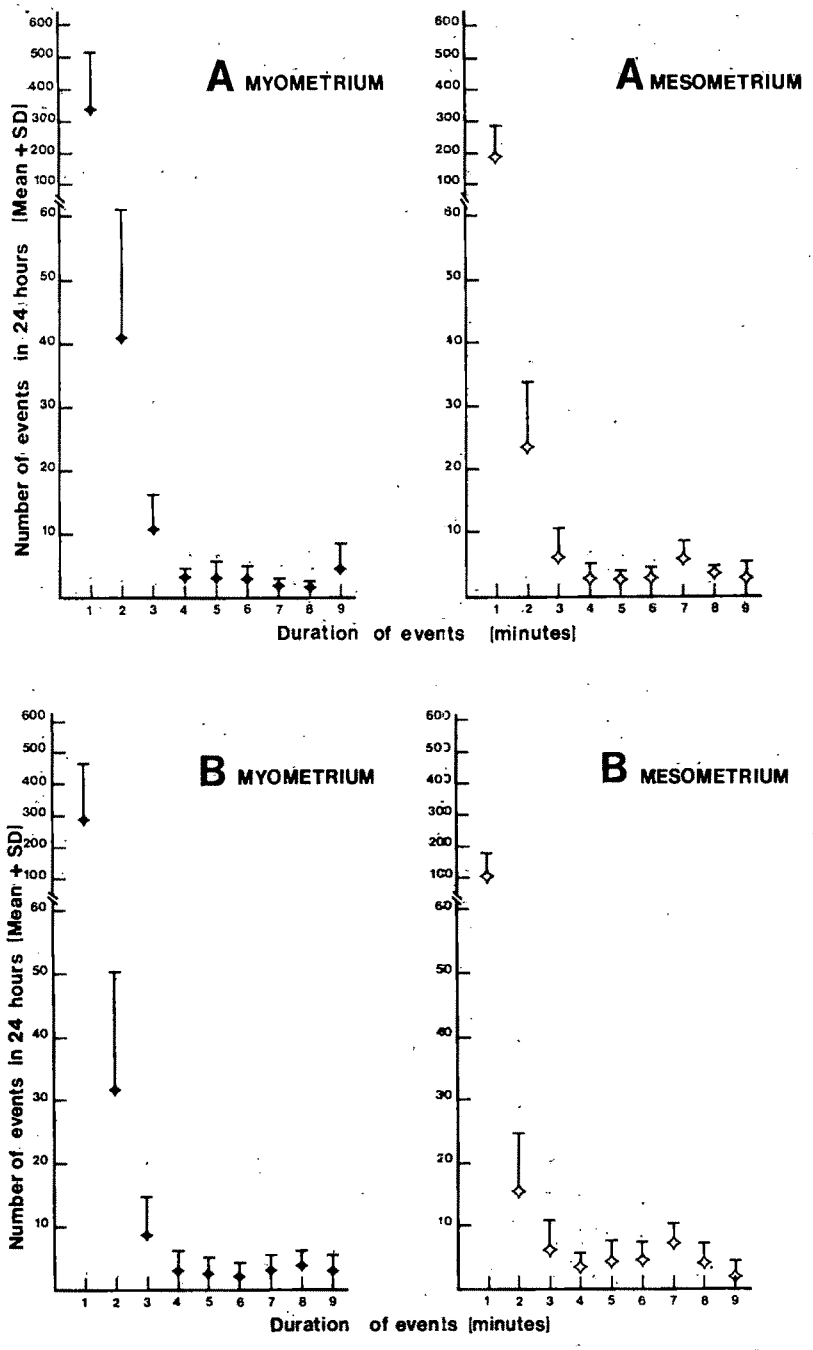


Fig. 2, *A* and *B*. Frequency distribution of electromyographic epochs of different durations in the myometrium and mesometrium in groups *A* and *B*. Epochs have been divided into 1-minute groups of burst duration.

to the fact that only a small portion of the myometrium is electrically active.

Preliminary data in the nonpregnant ovariectomized sheep show a late inhibition of electromyographic activity after exogenous oxytocin stimulation. It is possible to postulate an intrinsic mechanism within the muscle that will act to turn off contractile uterine activity. The extent of this inhibitory influence may depend on the intensity of the muscular contraction produced. The myometrium throughout gestation has more event activities <2 minute and the same contracture-type activity as the mesometrium. We hypothesize that the presence of endometrium in the nonpregnant ewe, and the fetal membranes in the pregnant

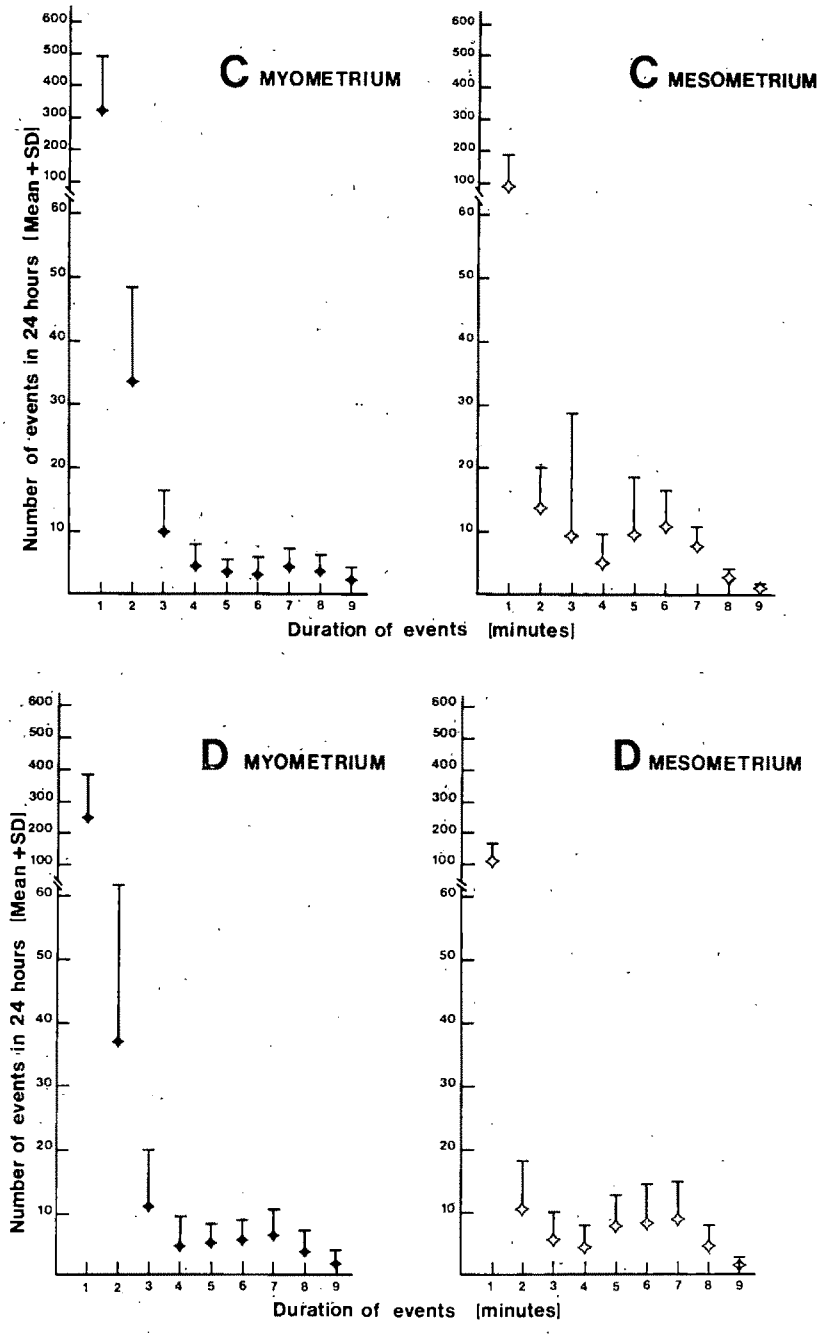


Fig. 2, *C* and *D*. Frequency distribution of electromyographic epochs of different durations in the myometrium and mesometrium in groups *C* and *D*. Epochs have been divided into 1-minute groups of burst duration.

ewe, may stimulate the myometrium by means of short-lived modulators that cannot reach the mesometrium.

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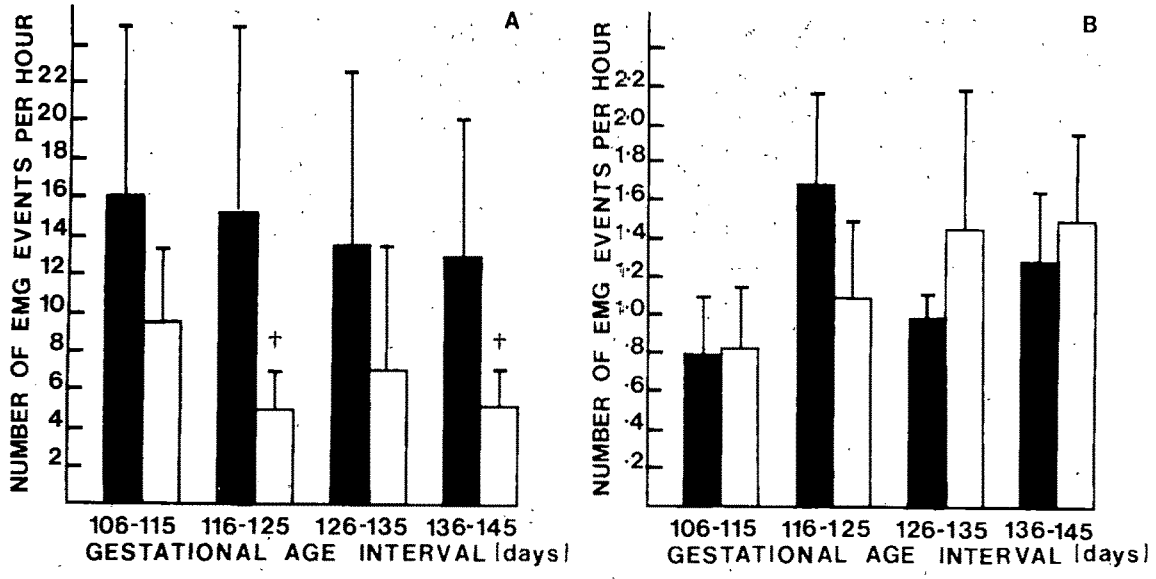


Fig. 3. Number of noncontracture electromyographic *(EMG)* events per hour (<180 seconds' duration) **(A)** and contracture electromyographic events per hour (>180 seconds' duration) **(B)** in the myometrium *(solid bars)* and mesometrium *(open bars)*. Data are mean ± SD (n = 4 to 7). †Myometrium significantly greater than mesometrium, $p < 0.05$.

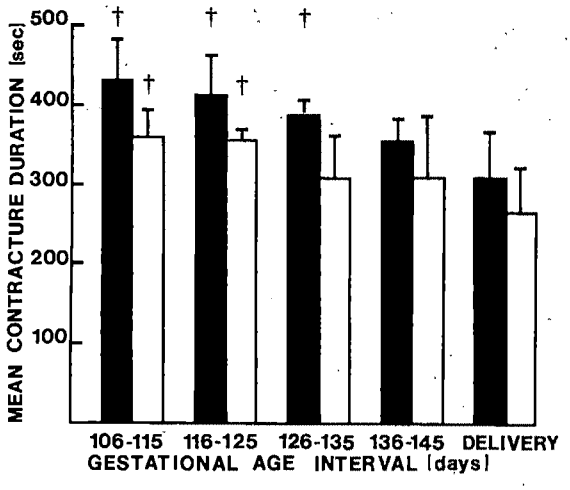


Fig. 4. Mean (± SD) contracture duration in the myometrium and the mesometrium between 106 days' gestational age and delivery (n = 4 to 7). †$p < 0.05$ when compared with the same tissue at delivery. At all gestational ages the mean contracture duration is longer in the myometrium *(solid bars)* than in the mesometrium *(open bars)*, except at 136 to 145 days ($p < 0.05$).

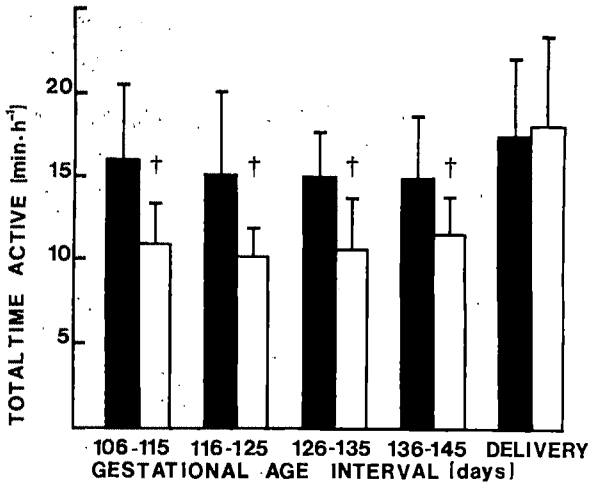


Fig. 5. Total time active (mean ± SD) for all events in the myometrium *(solid bars)* and mesometrium *(open bars)* between 106 days' gestational age and delivery. †$p < 0.05$ when compared with the same tissue at delivery.

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β-Adrenergic receptor agonist infusion increases plasma prostaglandin F levels in pregnant sheep

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Recent evidence of a stimulatory effect of β-adrenergic receptor agonists on prostaglandin production by human fetal membranes in vitro prompted us to investigate whether prostaglandins are increased during β-agonist infusion in vivo. On days 120 to 125 of gestation, blood samples were drawn from the aorta and vena cava of five crossbred ewes at 15-minute intervals for 1 hour before, during, and for 1 hour after a 3-hour infusion of isoproterenol (0.16 μg/kg/min) or normal saline solution. Plasma prostaglandin $F_{2\alpha}$ levels increased (177% ± 13% SEM over baseline; $p < 0.002$) in maternal aorta, while plasma 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ levels increased in both aorta and vena cava (354% ± 47% and 309% ± 75% over baseline, respectively; $p < 0.002$) during isoproterenol infusion but not during saline solution infusion. No change was seen in plasma prostaglandin E_2 levels. The increase in plasma stimulatory prostaglandins in the pregnant ewe during β-agonist infusion is compatible with the increased uterine activity observed after myometrial desensitization by continuous β-agonist infusion. (Am J Obstet Gynecol 1987;157:998-1003.)

Key words: β-Adrenergic receptor agonists, prostaglandins, fetal membranes

Infusion of β-adrenergic receptor agonists is widely used in the treatment of preterm labor. Although these drugs are unquestionably effective in inhibiting uterine activity acutely, data to support a benefit in the reduction of the incidence of preterm birth are lacking. We have previously reported that human myometrium exposed in vitro to isoproterenol exhibited initial cessation of contractile activity, but contractions returned with continuous exposure to the β-agonist.[1] We have also demonstrated that this phenomenon, termed desensitization, occurs in vivo in the sheep with continuous infusion of ritodrine or isoproterenol.[2] The loss of sensitivity of the myometrium to the relaxing effects of β-adrenergic agonists was dependent on the mode of administration. When the short-acting β-agonist isoproterenol was administered in an intermittent fashion, inhibition of both sheep[2] and human[1] myometrial activity could be maintained.

In in vitro[1] and in vivo[2] studies, we observed that, with the occurrence of desensitization and return of myometrial activity, both the amplitude and frequency of contractions appeared to be greater than that seen before β-adrenergic agonist infusion. A possible mechanism for this observed increase in uterine activity is the production of a uterine stimulant due to administration of the β-agonist. Because recent studies have demonstrated that β-adrenergic agents are capable of stimulating prostaglandin (PG) production by human intrauterine tissues in vitro,[3-5] we investigated whether an increase in PG production might occur in vivo in pregnant sheep exposed to β-adrenergic agonist in-

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Table I. Baseline data

	Isoproterenol		*Saline solution*	
$PGF_{2\alpha}$ (pg/ml)				
Aorta	247 ± 17	$p < 0.003$	278 ± 33	$p < 0.003$
Vena cava	516 ± 82		441 ± 36	
PGFM (pg/ml)				
Aorta	292 ± 44	NS	347 ± 33	NS
Vena cava	482 ± 103		423 ± 41	
PGE_2 (pg/ml)				
Aorta	306 ± 42	$p < 0.02$	329 ± 35	$p < 0.02$
Vena cava	469 ± 48		478 ± 43	
Progesterone (ng/ml)				
Aorta	8.6 ± 2.0	$p < 0.002$	7.5 ± 2.5	$p < 0.001$
Vena cava	21.8 ± 3.4		17.3 ± 2.5	
17β-Estradiol (pg/ml)				
Aorta	22.4 ± 2.4	NS	9.7 ± 0.7	$p < 0.02$
Vena cava	22.9 ± 2.6		14.5 ± 1.7	

Data are mean ± SEM calculated from the first five samples before isoproterenol or saline solution infusion. NS, Not significant.

fusion. If so, PGs may contribute to the increased uterine activity we observed after desensitization. We also measured plasma levels of PGE_2 and progesterone in these sheep, since infusion of a β-agonist into nonpregnant women during the luteal phase has been shown to decrease peripheral levels of these steroids,[6] which might in turn influence PG production. The demonstration of β-adrenergic stimulation of PG production would be an important effect to take into consideration when deciding on the use of these agents for the treatment of preterm labor.

Material and methods

Animals. With the animals under general anesthesia, surgery was performed on five pregnant, crossbred ewes between days 114 and 118 of gestation as previously described.[7] An amniotic cavity catheter was positioned for monitoring intrauterine pressure via a pressure transducer (Statham Instruments, Model P23, Oxnard, California) and stainless steel electrodes (Cooner Corporation, Chatsworth, California) were sutured into the myometrium of the pregnant uterine horn to record electromyographic activity. Intrauterine pressure and electromyographic activity were recorded on a Grass polygraph (Grass Instruments, Quincy, Massachusetts). Polyvinyl vascular catheters (VII Bolab, Lake Havasu City, Arizona) were inserted approximately 20 cm into the maternal femoral artery and vein, such that the tips of the catheters lay in the abdominal aorta and vena cava, respectively, 6 to 10 cm cranial to the branches of the uterine artery and the uteroovarian venous drainage. This catheter placement allowed sampling of peripheral blood (maternal aorta catheter) or blood enriched with uterine venous drainage (maternal vena cava catheter). The maternal external jugular vein was also catheterized with polyethylene tubing (PE100, Intramedic, Clay Adams, Parsippany, New Jersey) to permit peripheral infusions. The vascular lines were filled with sterile saline solution containing 4 U/ml sodium heparin and were flushed daily. The animals were given 7 days to recover from surgery and received food and water ad libitum.

Experimental protocol. On day 125 of gestation, between 8:00 and 10:00 AM, a 5 ml blood sample was drawn in a heparinized syringe from the maternal femoral artery and vein catheters every 15 minutes and stored immediately on ice. Within 15 minutes thereafter, cells were separated from plasma by centrifugation (4° C; 10,000 × g; 10 minutes). After 1 hour of baseline blood sampling, isoproterenol (0.16 μg/kg/min) or 0.9% sodium chloride was infused at a rate of 3 ml/hr for 3 hours. Blood sampling was continued for a further hour after the end of the infusion. The alternative infusion study was then done 48 to 72 hours later to give five isoproterenol studies and five saline solution studies. Plasma was stored at −20° C until assayed for prostaglandins, progesterone, or estradiol by radioimmunoassay.

Radioimmunoassay. The maternal artery and venous plasma concentrations of PGE_2, $PGF_{2\alpha}$, and 13,14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM) were determined by specific radioimmunoassay as previously described.[8] Briefly, plasma was acidified (to pH 3) with hydrochloric acid and the PGs were extracted with diethyl ether (8 vol). The ether extract was evaporated to dryness under nitrogen and the samples were reconstituted with phosphate buffered saline solution. Incubations were then performed with specific antisera[8] and tritiated prostaglandins (Amersham, Oakville, Ontario, Canada). The inter- and intrassay coefficients of variation for each PG were <11% and <9%, respectively. Estrogen and progesterone were extracted from plasma with the use of diethyl ether and petroleum ether, respectively, and assayed by radioimmunoassay as previously described.[9] The interassay and intra-assay coefficients of variation were 10% and 8% for progesterone and 7% and 6% for estradiol, respectively.

Data analysis. Because of considerable between-

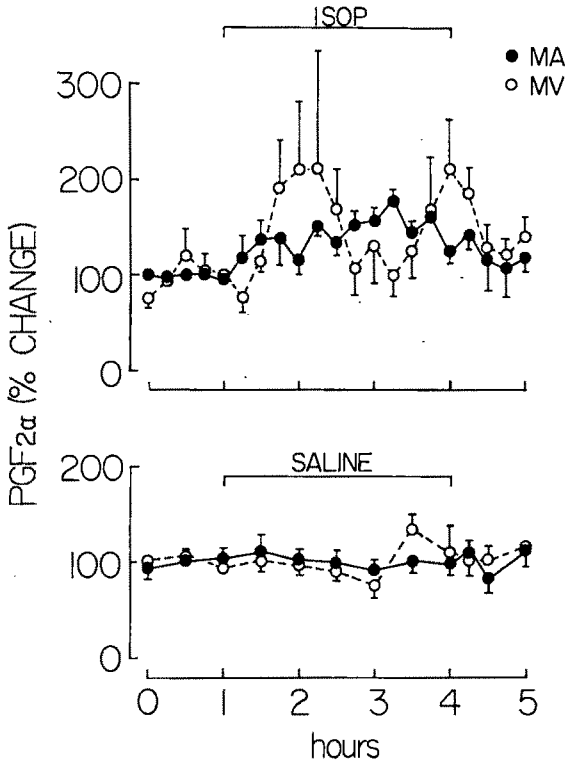


Fig. 1. Percent change from baseline in plasma $PGF_{2\alpha}$ levels in maternal vena cava (○) and maternal aorta (●) during isoproterenol *(Isop)* or saline solution infusions. A significant increase was seen in maternal aorta $PGF_{2\alpha}$ levels beginning at 105 minutes of infusion ($p < 0.002$). Maternal aorta $PGF_{2\alpha}$ levels returned to baseline by 15 minutes after isoproterenol infusion.

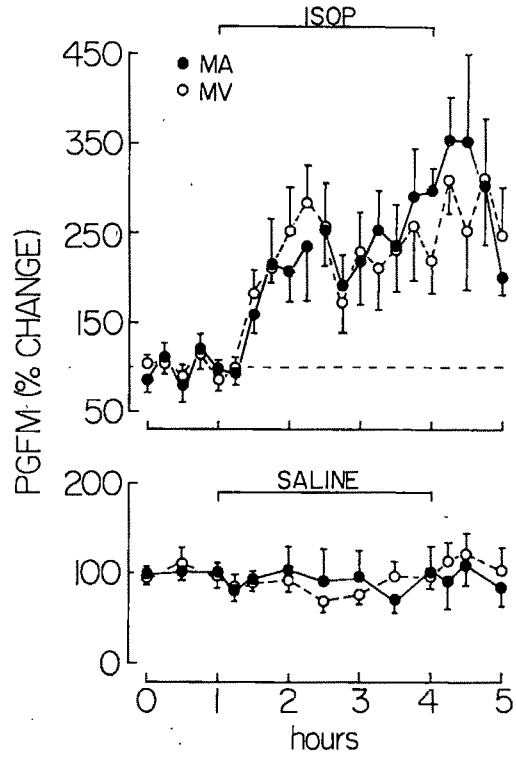


Fig. 2. Plasma PGFM levels shown as percent change from baseline during isoproterenol *(Isop)* and saline solution infusions. PGFM levels in maternal vena cava (○) and maternal aorta (●) increased significantly ($p < 0.002$) at 60 and 90 minutes, respectively. No significant change was seen during saline solution infusion.

animal variation in absolute PG levels, we have expressed the data as percentage change over pretreatment levels. Baseline concentrations of PGs were determined by calculating the mean of the five pretreatment samples; all samples were then expressed as a percentage of this mean. Data are shown as mean ± SEM throughout the article. To examine statistical significance between groups, data were subjected to analysis of variance and Duncan's multiple range test after determination of homogeneity of variance.

Results

Mean (±SEM) baseline levels of PGFM, $PGE_{2\alpha}$, PGE_2, progesterone, and estradiol were calculated from the first five plasma samples (Table I). Baseline values were higher in the uterine-enriched blood (maternal vena cava) than in peripheral blood (maternal aorta) for all hormones except PGFM, in which the values were equivalent.

$PGF_{2\alpha}$ levels increased in the maternal aorta during isoproterenol infusion, with the increase reaching significance ($p < 0.002$) after 105 minutes of infusion (Fig. 1). An apparent pulsatile pattern of vena cava $PGF_{2\alpha}$ levels was also observed, but did not result in a significant increase, likely because of variability in the frequency of $PGF_{2\alpha}$ pulses. No change in either aorta or vena cava $PGF_{2\alpha}$ levels was seen with infusion of 0.9% sodium chloride.

Plasma PGFM expressed as percent change from baseline is shown in Fig. 2 for isoproterenol- and saline solution–treated groups. During isoproterenol infusion, there was a progressive and significant ($p < 0.002$) increase in plasma PGFM levels in both maternal aorta and vena cava. This increase first reached significance after 1 hour of infusion in the vena cava and after 90 minutes in the aorta. The elevated levels continued after the end of the infusion, but appeared to begin to decline at 60 minutes after the infusion of isoproterenol. In contrast, no change was seen in PGFM levels during or after saline solution infusion.

PGE_2 levels did not change during isoproterenol infusion, except for a transient pulsatile release in the maternal vena cava immediately after the end of the infusion (Fig. 3). No change in aorta or vena cava PGE_2 levels was observed during or after saline solution infusion in the five sheep (data not shown).

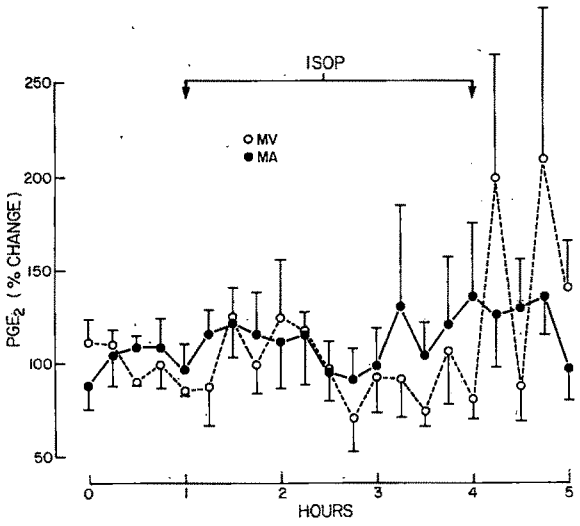


Fig. 3. Plasma PGE_2 levels expressed as percent change from baseline during isoproterenol *(Isop)* infusion. The levels in maternal vena cava (○) and maternal aorta (●) did not change significantly at any time during or after the infusion, although vena cava PGE_2 levels showed a marked pulsatile increase after the end of isoproterenol infusion.

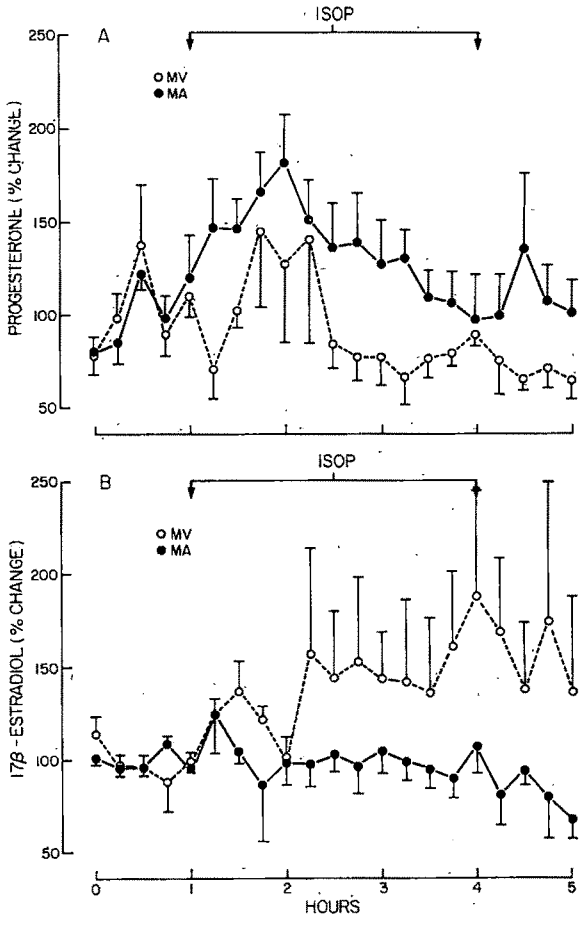


Fig. 4. Plasma progesterone **(A)** and 17-β-estradiol **(B)** levels in maternal vena cava (○) and maternal aorta (●) expressed as percent change from baseline. During isoproterenol infusion *(Isop)*, a significant ($p < 0.02$) but transient increase in plasma progesterone levels occurred initially in maternal aorta beginning at 45 minutes of infusion. No change was seen in the maternal vena cava progesterone levels or in 17-β-estradiol levels during isoproterenol infusion.

Plasma progesterone levels increased transiently ($p < 0.02$) after 45 minutes of isoproterenol infusion in the maternal aorta but not in the vena cava (Fig. 4, *A*). No change in progesterone levels was seen during saline solution infusion.

A variable response in vena cava plasma estradiol levels was seen in the five animals durring isoproterenol infusion (Fig. 4, *B*). In three animals, peak increases from 125% to 290% of baseline occurred, while two other animals demonstrated no change from baseline. No change was seen in maternal aorta estradiol levels during isoproterenol or saline solution infusions in any of the plasma samples.

These animals were not in labor at the time of any of the infusion studies, and quiescent uterine activity typical of a progesterone-dominant[10] environment was observed. Infrequent, low-amplitude increases in intrauterine pressure lasting 2 to 4 minutes and associated with an increase in electromyographic activity (contractures) occurred. There was no increase in uterine activity during either saline solution or isoproterenol infusion. However, a burst of electromyographic activity lasting approximately 5 minutes was often seen immediately after the end of isoproterenol infusion.

Comment

Our results demonstrate an increase in plasma levels of $PGF_{2\alpha}$ and its metabolite, PGFM, during infusion of the β-adrenergic agonist isproterenol in the pregnant ewe. The source of the elevated PG levels is likely the uterus or intrauterine tissues (that is, placenta or fetal membranes), because a significant gradient between the vena cava (uterine-enriched blood) and the aorta (peripheral blood) was observed for $PGF_{2\alpha}$. A similar gradient was not seen for PGFM, likely reflecting the major role of the lungs in the metabolism of $PGF_{2\alpha}$.[11, 12] Human fetal membranes and decidua have been shown to be a significant source of PG production. Skinner and Challis[13] demonstrated that dispersed cells of human amnion obtained at delivery produced more PGE_2 than $PGF_{2\alpha}$, while human decidual cells produced more $PGF_{2\alpha}$ than PGE_2. Chorionic cells appeared to play a mainly metabolic function, producing PGFM but not PGE_2 or $PGF_{2\alpha}$. Similar data have been reported by others.[4, 5]

To our knowledge, our study is the first to demonstrate increased plasma concentrations of stimulatory PGs in response to an in vivo infusion of a β-adrenergic

agonist during pregnancy. We chose isoproterenol for the experiments because of our previous data in sheep[2] demonstrating more rapid myometrial densensitization compared with ritodrine infusion. Our data contrast with in vivo reports in the rabbit[14] and baboon,[15] in which decreases in PGE and 6-keto-$PGF_{1\alpha}$ (the circulating metabolite of the inhibitory PG prostacyclin) levels, respectively, were found during infusion of the β-agonist ritodrine. These conflicting findings may reflect species differences or may be related to the type of β-agonist used. Ritodrine is a relatively selective β_2-receptor agonist, while isoproterenol has both β_1- and β_2-agonist activity. We are investigating the latter possibility. We found no rise in PGE_2 levels in our experiments, which differs from the in vitro results of β-adrenergic agonist (including isoproterenol) stimulation.[3-5] This finding may represent an interconversion of primary PGs in vivo. The presence of 9-keto PG reductase, which reduces PGE_2 to $PGF_{2\alpha}$, has been identified in ovine maternal and fetal blood.[16] The changes seen in estradiol and progesterone levels in our study are difficult to interpret. Estradiol levels increased inconsistently in the maternal vena cava during isoproterenol infusion, while those of progesterone increased transiently only in the maternal aorta. It is unlikely that these changes play a significant role in the alterations in PG levels that we observed. However, because fetal adrenal androgen may be a precursor of placental estradiol production,[17] it is possible that the rise in plasma estradiol levels in some sheep represents a fetal stress response to β-agonist infusion.

The pattern of PG elevations we report is similar to that occurring before and during adrenocorticotropic hormone–induced preterm labor in sheep. Olson et al.[18] demonstrated that $PGF_{2\alpha}$ and PGFM selectively increased in maternal vena caval and aortic plasma 16 hours or earlier before labor. No change was seen in PGE_2 levels. They concluded that the rise in stimulatory PGs occurred before the increased mechanical activity of the uterus during labor, suggesting that the rise in PGs was not a result of uterine activity. Rather, their findings implied that PGs promote the changes in contractility. Although no stimulatory effect of the PG elevation on myometrial activity was seen in the pregnant ewes we report, it is possible that long-term infusion may have lead to the onset of uterine contractions as observed by Olson et al. Alternatively, uterine activity in these sheep was suppressed by the high circulating levels of progesterone[10] normally present at day 120 of gestation. In preterm labor, in which uterine activity has already commenced, it is possible that myometrial sensitivity to PG action is enhanced and a stimulatory effect of the elevation of PGs may occur more rapidly.

At present, we cannot extrapolate our data to the human without measurement of PG levels in women during β-agonist infusion. However, if β-adrenergic agents also increase plasma PG levels in the human, our findings may be of interest clinically in view of the use of these drugs in the treatment of preterm labor. Recent observations, both in vitro[1, 19] and in vivo,[2, 20] have shown that continuous infusion of β-adrenergic agonists results in desensitization of the initial relaxing effect of these agents on the myometrium. Thus the current practice of continuous infusion of β-agonists for preterm labor, if prolonged to the point of desensitization, may lead to enhanced uterine sensitivity to the stimulatory effect of PGs on the myometrium. These factors may therefore contribute to the failure of β-agonist therapy in cases of clinical preterm labor.

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Nifedipine: Effects on fetal and maternal hemodynamics in pregnant sheep

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We investigated the effects of nifedipine, a calcium entry blocker, on the fetal and maternal circulation. Nifedipine was administered intravenously for 30 minutes to chronically instrumented pregnant ewes. Infusion of 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ resulted in a 30% to 50% increase in total and regional fetal cerebral blood flow to the brain stem, watershed, and subcortical regions ($p < 0.05$), without a significant change in fetal oxygenation or cardiac output. Infusion of 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ decreased uterine blood flow by 21% ($p < 0.001$) and fetal arterial oxygen content by 15% ($p < 0.01$), with no further increase in fetal cerebral blood flow. Maternal arterial pressure decreased and heart rate increased ($p < 0.001$) without variation of arterial blood gases. Significant plasma levels of nifedipine were detected in the fetal and maternal circulations. In view of the potential adverse effects on the fetus, further studies are needed before nifedipine is considered for use in human pregnancy. (AM J OBSTET GYNECOL 1987;157:1003-8.)

Key words: Nifedipine, calcium blocker, fetus, pregnancy, cerebral blood flow

Calcium entry blockers have been used effectively for the treatment of coronary artery disease, systemic hypertension, and supraventricular arrhythmias. Recent reports have also suggested potential therapeutic effects in several clinical conditions including premature labor,[1] dysmenorrhea, hypertrophic cardiomyopathy, preservation of the ischemic myocardium, peripheral vascular disease, cerebrovascular insufficiency, migraine headache, pulmonary hypertension, asthma, and esophageal spasm.[2]

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Little information is currently available regarding the effects of these agents on the fetus or during pregnancy. Nifedipine, a dihydropyridine calcium entry blocker, has been shown to effectively control the hypertension accompanying preeclampsia and to exert a beneficial tocolytic effect in cases of premature labor without apparent adverse effects on either the mother or the newborn.[1, 3] However, recent animal studies have shown potentially hazardous effects reflected by a decrease in uterine blood flow and fetal oxygenation.[4, 5] Ducsay et al.[6] reported a marked tocolytic action of nifedipine accompanied by significant fetal acidosis and hypoxemia in pregnant rhesus monkeys. Our purposes were to investigate both maternal and fetal hemodynamic effects during maternal administration of nifedipine and to measure possible variations in fetal regional blood flow. We were particularly interested in

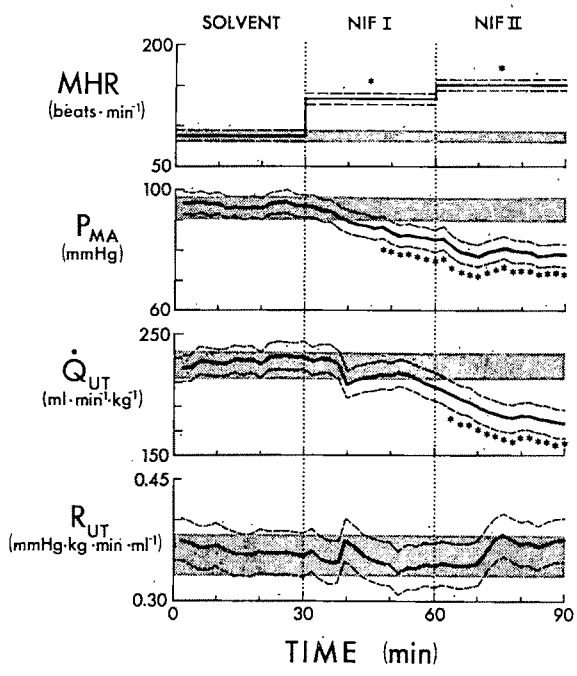


Fig. 1. Average maternal heart rate *(MHR)*, mean arterial pressure (P_{MA}), uterine blood flow ($\dot{Q}_{UT}$), and uterine vascular resistance (R_{UT}) during solvent and nifedipine infusions at 5 and 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$. The horizontal *gray band* shows the 95% confidence interval of control values. All values are expressed within 95% confidence limit *(dashed lines)*. *$p < 0.05$ relative to control.

determining whether nifedipine was associated with changes in fetal oxygenation and the possible mechanisms of this response.

Material and methods

Animal preparation. We studied eight pregnant ewes of mixed-western breed with a gestational age of 123 to 138 days (term 147 days). All fetuses were singletons with a mean (±SD) weight of 3.2 ± 0.9 kg.

General anesthesia was induced with 20 ml of intravenous sodium thiamylal and was maintained, after intubation, by inhalation of 1.0% to 1.5% halothane and 30% nitrous oxide in oxygen. Surgery was performed as described by Lotgering et al.[7] in previous experiments in our laboratory. We placed catheters in the maternal descending aorta and inferior vena cava through a femoral artery and vein. Using a left flank retroperitoneal approach, we placed an 8 mm inside diameter electromagnetic flow probe (Zapeda Instruments, Seattle, Washington) around the common internal iliac artery. The uterus was subsequently exposed and, after a hysterotomy incision, catheters were introduced into the fetal ascending and descending aorta and inferior and superior vena cava. A catheter was placed in the amniotic cavity and two catheters were placed in the uterine vein. All incisions were closed and catheters led subcutaneously to a pouch on the ewe's flank. Postoperative care consisted of daily administration of 500 mg ampicillin injected into the amniotic cavity and 2 ml Combiotic (Pfizer Inc., New York, New York) administered intramuscularly to the ewe. Fetal arterial blood gases were measured daily to ascertain fetal well-being.

Experimental protocol. We allowed the animals to recover for 3 to 4 days after surgery. A 0.5 $mg \cdot ml^{-1}$ nifedipine solution was prepared the day of the experiment by dissolving 0.050 gm nifedipine (Pfizer Inc.) in 15 gm ethyl alcohol and 15 gm polyethylene glycol with water added to 100 ml solution. Because of its sensitivity to light, we handled nifedipine away from light sources and wrapped the infusion syringe with aluminium foil.

The experiment consisted of four consecutive phases, each lasting 30 minutes: control period, solvent infusion ($0.02 \text{ ml} \cdot kg^{-1} \cdot min^{-1}$), and nifedipine infusion at both 5 and 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$. The infusions were administered through the maternal inferior vena cava by a Harvard Instruments pump. During the 2-hour experimental period, the following parameters were continuously recorded on a polygraph (Gould Inc. Model 2002, Cleveland, Ohio): maternal mean arterial pressure and heart rate, uterine venous pressure and uterine arterial flow rate, fetal heart rate and mean arterial pressure, and amniotic fluid and superior and inferior vena cava pressures. The fetal pressures were corrected for amniotic fluid pressure. Mean values were displayed every 10 seconds and stored on computer disk (Texas Instruments 990/10). During each phase of the experiment, blood samples were drawn every 10 minutes from the maternal descending aorta and uterine vein and from the fetal ascending aorta for pH, blood gas analysis (Radiometer Copenhagen, Denmark, type ABL 2d), and hemoglobin estimation (Radiometer America Inc., Cleveland, Ohio). Maternal and fetal blood samples were taken at 25 minutes of the last two phases for nifedipine plasma assays by gas chromatography (Harris Laboratories, Lincoln, Nebraska).

We measured fetal cardiac output and regional organ blood flow during each of the four phases of the experiment with radioactive microspheres 15 ± 1 μm in diameter labeled with scandium 46, chromium 51, ruthenium 103, and gadolinium 153 (New England Nuclear, Boston, Massachusetts). Microsphere infusions were peformed during the last 5 minutes of each phase. The ewe was killed at the end of the experiment by the administration of 20 ml of euthanasia solution (National Laboratories, Summerville, New Jersey). Fetal organs and placental cotyledons and membranes were weighed, ashed, and counted for radioactivity. The separated ashed organs were: kidneys, adrenal glands, heart, lungs, gastrointestinal tract, spleen, liver,

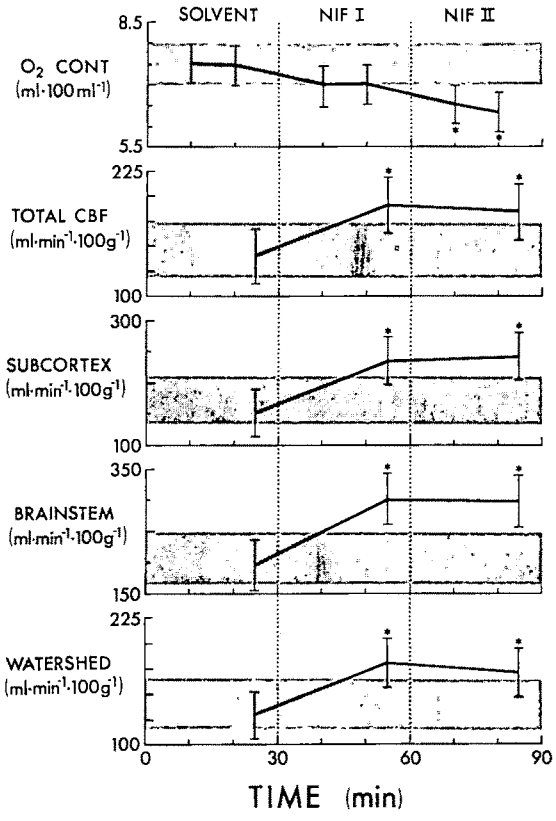


Fig. 2. Fetal ascending aorta O_2 content *(O_2 CONT)* and total and regional cerebral blood flow *(CBF)* during solvent and nifedipine infusions at 5 and 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$. The horizontal *gray band* shows the 95% confidence interval of control values. All data are expressed within 95% confidence limit *(vertical bars)*. *$p < 0.05$ relative to control.

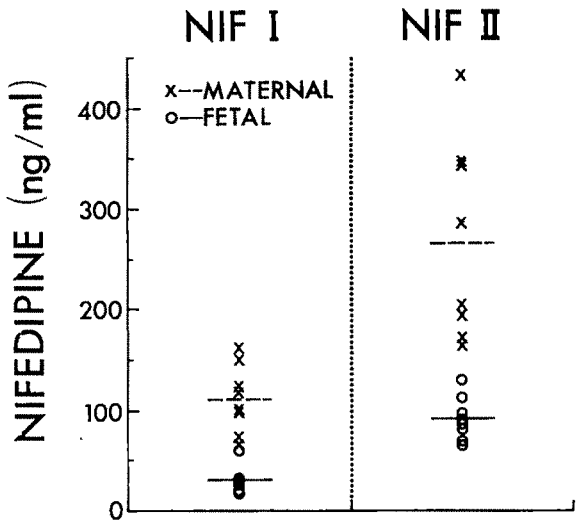


Fig. 3. Fetal and maternal plasma nifedipine levels at the end of 30-minute infusions of 5 *(NIF I)* and 10 *(NIF II)* μg/kg/min. *Horizontal lines* show mean values.

brain, total carcass, and placenta. The brain was dissected into five regions: brain stem, subcortex, gray matter, white matter, and watershed between the anterior-middle and middle-posterior cerebral arteries. Absolute fetal organ blood flow and cardiac output were computed from relative concentrations of radioactivity.[8] We calculated fetal arterial oxygen content, fetal cerebral vascular resistance, uterine oxygen consumption, and uterine vascular resistance during the different phases of the experiment. Vascular resistance was calculated as mean arterial pressure minus venous pressure divided by organ blood flow.

Statistical analysis. Unless otherwise stated, values are expressed as means ± SEM. We tested differences between means of control values and experimental measurements by two-way analysis of variance and Duncan's multiple-range test. A p value <0.05 was considered to indicate significance.

Results

Maternal and fetal hemodynamics. Maternal hemodynamic responses during solvent and nifedipine infusions are shown in Fig. 1. There were no significant changes in heart rate or mean arterial blood pressure during solvent infusion. Maternal heart rate increased and blood pressure decreased progressively during nifedipine infusion ($p < 0.001$). During the control period, the average uterine venous pressure, oxygen consumption and vascular resistance were 14.1 ± 1.6 mm Hg, 7.0 ± 0.8 ml · min^{-1} · kg^{-1}, and 0.35 ± 0.02 mm Hg · kg · min · ml^{-1}, respectively; none of these values showed significant variation with either solvent or nifedipine infusion. The uterine blood flow decreased by 21%, from a control value of 224 ± 7 to 177 ± 11 ml · min^{-1} · kg^{-1} at the end of the experiment ($p < 0.001$). The control fetal mean arterial pressure and heart rate were 47.7 ± 2.8 mm Hg and 170 ± 9 bpm, respectively, with no change observed during the experiment.

Maternal and fetal blood gases. Maternal blood gas values during the control period were: Pa_{O_2}, 96.2 ± 2.9 mm Hg; Pa_{CO_2}, 37.7 ± 1.2 mm Hg; and pH, 7.45 ± 0.01; these values did not change significantly during the study. Fetal Pa_{O_2} fell progressively during nifedipine infusion without reaching statistical significance (Table I). However, fetal arterial O_2 content (Fig. 2) and O_2 saturation (Table I) significantly decreased at the end of nifedipine infusion ($p < 0.01$ and $p < 0.001$, respectively).

Fetal organ blood flow. Fetal organ blood flows tended to increase with nifedipine, but only brain blood flow reached statistical significance. During the control period, blood flow to the brain, heart, and adrenal glands was 146 ± 16, 228 ± 26, and 377 ± 68 ml · min^{-1} · 100 gm^{-1}, respectively; the heart and ad-

Table I. Fetal ascending aorta blood gas values

	Control	*Solvent*	*Nifedipine*	
			5 μg · kg⁻¹ · min⁻¹	*10 μg · kg⁻¹ · min⁻¹*
Pa_{O_2} (mm Hg)	23.0 ± 1.2	23.3 ± 1.3	22.4 ± 1.3	21.1 ± 1.2
O_2 saturation (%)	54.0 ± 3.6	53.2 ± 3.9	49.2 ± 3.6	44.0 ± 3.3*
Pa_{CO_2} (mm Hg)	49.6 ± 1.0	49.0 ± 1.2	49.7 ± 1.5	51.6 ± 1.0
pH	7.34 ± 0.01	7.33 ± 0.01	7.31 ± 0.02	7.31 ± 0.01†

Data are presented as mean ± SEM.
*$p < 0.001$.
†$p < 0.01$.

renal glands manifested an increase, relative to control, with solvent as well as nifedipine. Control fetal cardiac output was 496 ± 56 ml · min⁻¹ · kg⁻¹; there was no significant variation during other phases of the experiment. Fig. 2 shows the variations in total and regional cerebral blood flow. Control blood flows to the brain stem, subcortical, and watershed regions were 204 ± 25, 171 ± 19, and 139 ± 19 ml · min⁻¹ · 100 gm⁻¹, respectively. The brain stem showed a 50% increase in flow during both nifedipine infusions, and a 30% to 42% increase in flow was observed in subcortical and watershed areas. Control blood flows to gray matter and white matter were 141 ± 16 and 108 ± 14 ml · min⁻¹ · 100 gm⁻¹, respectively; these values did not vary significantly after nifedipine infusion. No significant change in cerebral blood flow was observed with solvent infusion. During the control period, the average cerebral vascular resistance, calculated from five animals for which complete data were available, was 0.029 ± 0.004 mm Hg · kg · min · ml⁻¹; it decreased by 24% ($p < 0.05$) during the first nifedipine infusion. There was no significant change in this parameter during other phases of the study.

Nifedipine plasma levels. Fig. 3 shows individual and average plasma levels of nifedipine. At the end of the first infusion (5 μg · kg⁻¹ · min⁻¹), average fetal and maternal plasma levels were 29 ± 5 and 110 ± 12 ng · ml⁻¹, respectively; at the end of the second infusion (10 μg · kg⁻¹ · min⁻¹), fetal and maternal levels were 91 ± 8 and 267 ± 35 ng · ml⁻¹, respectively.

Comment

Our study has shown that nifedipine significantly altered maternal and fetal hemodynamics. Nifedipine infusion resulted in a progressive decrease in maternal mean arterial pressure and uterine blood flow. Because uterine vascular resistance did not vary significantly, the fall in uterine blood flow could be explained entirely by the hypotensive effect of nifedipine. In contrast to our findings, Veille et al.[9] did not observe any significant change in uterine blood flow in response to bolus injections of 20 to 40 μg · kg⁻¹ and described only a transient fall in maternal arterial blood pressure in the first minute after the boluses. Although our data differ from those of Veille et al., both studies indicate that the decreased uterine blood flow associated with nifedipine occurs only with a significant decrease in maternal arterial pressure. In other studies of calcium entry blockers, nicardipine[4] and verapamil[10] significantly decreased uterine blood flow in pregnant rabbits and ewes. Contrary to these findings, nifedipine has been shown to increase uterine blood flow in the nonpregnant rat,[11] which could be attributed to a decrease in uterine vascular resistance. These data suggest that the vasodilator effect of nifedipine on the uterus is not present during pregnancy. Whether these observations are related to the overall physiologic vasodilation that occurs in pregnancy or to the anatomy of placental vasculature is still to be determined. Our data do not differentiate between myoendometrial and placental flow. Parisi et al.[12] have shown that nicardipine administered during angiotensin II infusion caused vasodilation in the myoendometrium, but no change in placental resistance occurred. Whether such a difference in response might have occurred in our experiment is not known; however, it is probably unlikely because total uterine resistance did not change.

Nifedipine infusion resulted in a trend toward fetal hypoxemia and acidemia. These changes were significant at higher doses (10 μg · kg⁻¹ · min⁻¹) and agree with the findings of Ducsay et al.,[6] who reported a dramatic decrease in fetal arterial pH and partial O_2 pressure (from 21.7 to 14.2 mm Hg) during continuous nifedipine infusion at 3 μg · kg⁻¹ · min⁻¹ in pregnant rhesus monkeys. In all these reports, there was no variation in maternal arterial pH or blood gases. In our study, fetal hypoxemia was most probably related to the 21% fall in uterine blood flow. In the fetal lamb, Skillman et al.[13] observed a decline in arterial Po_2 and O_2 content with reductions of 24% to 63% in uterine blood flow. They concluded that the amount of fetal hypoxia was a direct consequence of the degree of restriction in uterine blood flow.

Our data and those of other investigators[6] did not

show any significant fetal hypotension with nifedipine. The mechanism of this difference from the adult response is uncertain; whether it is dose related or a result of opposing effects to nifedipine and hypoxemia on fetal resistance vessels needs to be determined.

Little information is currently available on the effects of calcium entry blockers on fetal organ blood flow. With the use of nicardipine, Holbrook et al.[4] reported increases in blood flow to the fetal brain and myocardium of 61% and 183%, respectively. In our experiment, only total and regional cerebral blood flows were increased by nifedipine. The increases in myocardial and adrenal blood flows were not significant because similar changes occurred during solvent infusion. The increase in total cerebral blood flow was associated with a significant drop in cerebral vascular resistance during the first infusion; the unsustained decrease in resistance during the high-dose infusion was probably due to individual variability and to the limited number of animals. Several calcium entry blockers, particularly nimodipine and nifedipine, have been shown to vasodilate cerebral vessels in vitro and to increase cerebral blood flow in vivo.[14, 15] In most of these studies, a 50% to 100% increase in both total and regional cerebral flow was observed during infusions of 1 to 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$. These findings have been reported in a variety of species, in adult nonpregnant as well as newborn animal models.[16]

It is possible that during the 10 $\mu g \cdot min^{-1} \cdot kg^{-1}$ nifedipine infusion, fetal hypoxemia contributed to the increase in cerebral blood flow. Longo et al.[8] showed that hypoxemia in sheep increased blood flow to the fetal brain, heart, and adrenal glands. In our study, a nifedipine infusion of 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ decreased O_2 content 15% and was associated with a 29% increase in cerebral blood flow. However, the maximum increase in fetal cerebral blood flow occurred at the lower infusion rate (5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and was not accompanied by any appreciable change in oxygenation. Furthermore, nifedipine infusion at 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ did not result in any additional increase in cerebral blood flow, or blood flow to any other fetal organ, although the fetal arterial O_2 content was significantly decreased. Thus our data indicate that the maximal increase in fetal cerebral blood flow occurred at lower doses without any significant change in either uterine blood flow or fetal oxygenation, suggesting that nifedipine had a direct cerebral vasodilatory effect.

These dose-related effects might have potentially important clinical implications, because significant tocolytic effects appear at relatively low doses in the range of 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$.[3] However, more studies are needed, perhaps with fetal infusions of nifedipine, to differentiate between direct fetal effects and changes secondary to alterations in maternal circulation. We emphasize that our findings apply mainly to conditions during normal pregnancy. In cases of hypertension accompanying pregnancy, a clinical trial of nifedipine[1] apparently did not result in any adverse effect. In contrast, the administration of nicardipine to 15 hypertensive ewes resulted in fetal hypoxia, acidosis, and the alarming death of five fetuses.[12]

It is difficult to interpret our findings of increased cerebral blood flow in relation to the well-being of the fetus. In the term fetus, cerebral germinal matrix vasculature is well developed and unlikely to bleed in response to increases in flow or perfusion pressure. In the preterm fetus, periventricular ischemia contributes to the development of hemorrhage, and reducing such ischemia should decrease the risk of its complications. However, in cases of hyperperfusion, serious concerns arise because of the association with intracranial bleeding.

Nifedipine crossed the placenta with significant levels detected in the fetal circulation. Fetal-to-maternal plasma concentration ratios were 0.3 and 0.4 during infusions, resulting in fetal plasma levels of 29 ± 4 and 91 ± 8 ng $\cdot$ ml^{-1}. Based on adult human studies, sublingual administration of 10 to 40 mg nifedipine to healthy volunteers resulted in plasma levels ranging between 28.7 ± 3.7 and 138.7 ± 43.7 $\mu g \cdot ml^{-1}$.[17] Ulmsten[1] used similar doses to treat normotensive and hypertensive patients with premature labor. We suggest that the plasma levels in our study corresponded to optimal nifedipine dosages in sheep.

In conclusion, during the intravenous administration of nifedipine to the pregnant ewe, uterine blood flow and fetal arterial oxygen content decreased while fetal cerebral blood flow increased. This increase in cerebral blood flow preceded any significant change in fetal oxygenation. Nifedipine crossed the placenta and significant levels were detected in the fetal circulation. In addition to its antihypertensive and tocolytic effects demonstrated in previous studies, we speculate that nifedipine may play a therapeutic role in cases of fetal distress and compromised cerebral circulation. However, because of the potential deleterious effects of nifedipine on the fetus, further studies are needed before this agent is considered for use in human pregnancy.

The technical assistance of Lela Spears, Dong Quan-Sheng, and Steven Dale is greatly appreciated. We thank Pfizer Inc. for providing nifedipine powder and Harris Laboratories for performing nifedipine plasma assays.

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CORRESPONDENCE

Absence of clomiphene and/or active metabolites in blood of clomiphene citrate–treated patients during organogenesis: Determination by radioreceptor assay

To the Editors:

The association between intrauterine exposure of female fetuses to diethylstilbestrol and the later occurrence of uterine, cervical, and vaginal anomalies, as well as clear cell adenocarcinoma of the cervix and the vagina, is well documented.[1-3]

Since the diethylstilbestrol molecule is structurally related to the clomiphene molecule, the presence of clomiphene or clomiphene metabolite during estrogen-sensitive organogenesis may interfere with normal development of those organs, resulting in the previously mentioned diseases. Studies with carbon 14–labeled clomiphene citrate have shown that clomiphene is excreted principally in the feces.[4] An average of 51% of the administered dose was excreted after 5 days. The appearance of carbon 14 in the feces 6 weeks after administration suggests that the remaining drug and/or metabolites are slowly excreted from a sequestered enterohepatic recirculation pool.

Such studies do not necessarily indicate that the radiolabeled compounds measured are biologically active, that is, capable of binding to the estrogen receptor in estrogen target cells. Binding to the estrogen receptor is thought to be a requirement of a compound that has estrogenic and/or antiestrogenic activity.

A simple radioreceptor assay suitable for measuring clomiphene and its active metabolites simultaneously in serum of patients undergoing clomiphene treatment was developed by our group.[5] The assay is based on the competitive replacement of radiolabeled estradiol in the estrogen receptor of the rat uterus by the active ligands present in the serum.

The amount of ligand extracted from the serum was expressed as estradiol-binding equivalent, that is, their equivalent in pycograms of estradiol.

To exclude the contribution of endogenous estrogens, three volunteer women with amenorrhea with no endogenous estrogens were selected for the study.

After the usual treatment scheme of clomiphen, estradiol-binding equivalent concentration rose and reached its highest levels (ranging from 2000 to 7000 pg/ml) about 12 hours after the last drug was administered. Thereafter estradiol-binding equivalent levels declined gradually.

On day 54 after cessation of clomiphene therapy, no clomiphene and/or metabolites (estradiol-binding equivalent equaled pretreatment levels <80 pg/ml) were found in the patient's sera.

In conclusion, the observation that on day 54, the assumed time of estrogen-sensitive organogenes in the conception cycle, no estrogen receptor–binding ligands were detected is reassuring regarding possible late adverse effects of the drug. However, differences in the metabolic clearance rate of the drug in euestrogenic and pregnant women could not be excluded. Furthermore, since the drug has only been used for 20 years, final conclusions on the long-term safety to the offspring will have to be delayed until sufficiently large surveys on postpubertal offspring have been concluded.

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Spontaneous fetal seizures in utero

To the Editors:

The recent report by Conover et al. (Antenatal diagnosis of fetal seizure activity with use of real-time ultrasound. AM J OBSTET GYNECOL 1986;155:846-7.) of the first two cases of spontaneous fetal seizures in utero has reinforced my belief that such seizures occur more frequently than would appear from a review of the literature. I describe a case of what I believe is intrauterine fetal seizures causing a characteristic artifact on direct fetal scalp electrode cardiotocography.

A 25-year-old primigravida had labor induced by prostaglandin E_2 vaginal pessary because of moderate preeclampsia at term. During labor she was sedated with chlormethiazole and was given an epidural analgesic. A normal infant weighing 3.1 kg was easily delivered with Neville-Barnes forceps because of delay in the second stage of labor. The Apgar score was 7 at 1 minute and 8 at 5 minutes. Throughout labor the fetal heart was monitored by direct scalp electrode cardiotocography with a single helix scalp electrode and a Hewlett-Packard 8030A cardiotocograph. The fetal

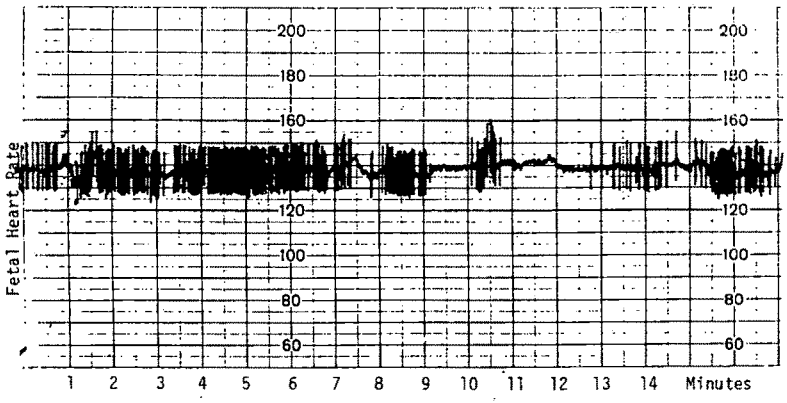


Fig. 1. Fetal heart rate tracing.

heart tracing was normal, with intermittent episodes of artifacts resembling a railway line (Fig. 1). At 24 hours of age, the infant started to have seizures that required phenobarbitone, paraldehyde, chloral hydrate, and eventually diazepam to control. A computerized tomography scan performed on the fourth day of life showed extensive infarction of the right cerebral hemisphere. A full investigation did not reveal any other etiologic factors. The infant has a left flacid hemiparesis at 2 months of age.

I believe that the artifacts seen on the cardiotocograph represent intrauterine fetal seizures. My reasons are as follows.

There was no evidence of perinatal asphyxia, which is the common cause of early neonatal convulsions. The forceps delivery was straightforward, and the infant's condition at birth was good.

Intrauterine fetal seizures would be expected to cause intermittent electrical discharges of an amplitude in the range of 100 to 500 μV. This is within the sensitivity range of the Hewlett-Packard 8030A (30 μV to 3 mV). Furthermore, when transcutaneous electrical nerve stimulation is used for pain relief during labor, a burst of high-frequency electrical discharge between electrodes attached to the maternal skin causes the same artifact on direct cardiotocography.

The episodic and recurrent nature of these artifacts form a pattern that is consistent with fetal convulsions and is unlikely, in my opinion, to be from poor contact of the scalp electrode.

I have seen one other case of cardiotocographic artifacts under similar clinical circumstances and an almost identical appearance of the infant's brain on the computerized tomography scan. Unfortunately, that cardiotocograph has been lost.

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Human ovarian oxytocin and steroid hormones

To the Editors:

We read with great interest the article by Dawood and Khan-Dawood (Human ovarian oxytocin: its source and relationship to steroid hormones. AM J OBSTET GYNECOL 1986;154:756-63) that describes the source of human ovarian oxytocin and its relationship with steroid hormones. The authors conclude that oxytocin is present in and is probably produced by the human corpus luteum.

This conclusion is, however, clouded by the analytic procedures used in these investigations. For example, in the figure showing reverse-phase high-pressure liquid chromatography (Fig. 1, p. 757) and in the Methods section, no mention is made of the sensitivity of their high-pressure liquid chromatography system. How many micrograms (or nanograms) of authentic oxytocin were used as the standard? The authors also fail to report the wavelength used in their detector. This is important because detection wavelength determines sensitivity to some extent. This aspect is vital in view of the fact that a peak of absorbance apparently corresponding to oxytocin appears on the high-pressure liquid chromatography tracing from the corpus luteum extract. Is this peak oxytocin? It seems very unlikely because the corpus luteum only contains, at most, 30 ng/gm of luteal tissue (as shown in Fig. 3, p. 758) at its peak during the luteal phase; the quantities of peptides (i.e., authentic oxytocin) used to calibrate high-pressure liquid chromatography systems are usually measured in micrograms.

As a sidelight, ref. 9, which refers to the synthesis of oxytocin from labeled precursor, should be ref. 8. In the Swann et al. paper (ref. 8), no tritium-leucine was used; sulfur 35–cysteine was the label employed.

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Reply

To the Editors:

We are pleased that Drs. Auletta and Flint are interested in our paper "Human ovarian oxytocin: its source and relationship to steroid hormones" (AM J OBSTET GYNECOL 1986;154:756-63). For this study, reverse-phase high-pressure liquid chromatography was performed on a 3.9 by 30 mm Bondapak C_{18} column (Waters Associates, Milford, Mass.) by gradient elution with 0.1% trifluoroacetic acid and water (solvent A) and 70% acetonitrile, 29.9% water and 0.1% trifluoroacetic acid (solvent B) at a flow rate of 1 ml/min. The elution positions of both the standard oxytocin and the corpus luteum extract were monitored by absorption at 214 nm. Under these conditions, the minimum amount of oxytocin standard that can be detected is 20 ng at a AUFS (absorbance units full scale) setting of 0.01. The chromatogram shows that a peak of oxytocin standard (250 ng) at a retention time of 14.86 minutes and the quantity of radioimmunoassayable oxytocin in the corpora lutea extracts that was chromatographed are equivalent to 320 ng of oxytocin. The extract of tissue that was chromatographed was a pool from 12 corpora lutea. Each corpus luteum extract was first assayed by radioimmunoassay, and the extracts were then pooled and reassayed for oxytocin. Since the publication of this paper, we have obtained consistently similar findings with reverse-phase high-pressure liquid chromatography of extracts of our on-going corpora lutea collection. Therefore we are confident that the similar retention times and peaks obtained with the corpora lutea and the pure oxytocin standard represent oxytocin.

We agree with Drs. Auletta and Flint that ref. 9 referring to the synthesis of oxytocin from labeled precursor should be ref. 8 and that ^{35}S-cysteine and not ^{3}H-leucine was employed. We regret the typographic errors and thank Drs. Auletta and Flint for pointing them out.

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Teen pregnancies

To the Editors:

Drs. Hollingsworth and Felice (Teenage pregnancy: A multiracial sociologic problem. AM J OBSTET GYNECOL 1986;155:741-6) cite decreasing teen birth rates to support their conclusions about teen pregnancy rates. The two are incomparable. Not all teen pregnancies result in birth—thousands end in abortion every day. This error in the article prohibits the authors from the conclusion that teen pregnancies are not increasing.

Table I. Pregnancy rate, abortion rate, and birth rate per 1000 women, ages 15 to 19 years, for all races, 1979 to 1983

	1980	*1983*
Pregnancy rate, age at outcome*	111.2	109.9
Abortion rate	42.9	43.5
Birth rate	53.3	51.7

*Includes estimated numbers of pregnancies ending in spontaneous abortions and stillbirths. These data present additional evidence that both pregnancy and birth rates have declined in teenagers.

Gregory R. Roush
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Reply

To the Editors:

We thank Gregory R. Roush for his letter and comment that birth rates and pregnancy rates are not comparable. His observation is correct, and not all teen pregnancies result in birth.

In our paper we relied on public birth and census figures.[1]

Henshaw,[2] who used well-established estimate formulas for pregnancy rates, has recently reported that from 1978 to 1983, the pregnancy rates for nonwhite 15- to 19-year-old teens decreased whereas pregnancy rates for white teenagers have increased. Table I shows the decrease in pregnancy rate and birth rate from 1980 to 1983 with very little change in abortion rate. After rising during the 1970s, the adolescent pregnancy rate peaked around 1980 to 1981 and fell slightly in 1982 to 1983.

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REFERENCES

1. Hollingsworth DR, Felice ME. Teen-age pregnancy: a multiracial sociologic problem. AM J OBSTET GYNECOL 1986; 155:741-6.
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Have Liley charts outlived their usefulness?

To the Editors:

I read with great interest the article "Have Liley charts outlived their usefulness?" by Nicolaides et al.

that appeared in the July issue (Am J Obstet Gynecol 1987;155:90-4).

Rather than relying on amniotic fluid specimens to manage high-risk, Rh-sensitized pregnancy, they advocate the use of fetal hemoglobin levels. On the several occasions when I heard Dr. Liley present his material during visits to the United States, I seem to remember him always saying that the tables were not good before the twenty-eighth week. In his original 1963 article "Errors in the Assessment," when he presented his table of probabilities the table began at week 28 of gestation and concluded in week 40 of gestation. Dr. Liley said to me on at least one occasion that there was not enough experience in his controlled studies to make predictions before 28 weeks and not after 40 weeks.

Over the years several authors have persisted in extending Liley's diagonal lines back in space so that predictions could be made without the outcome of pregnancy at 22, 24, or even 26 weeks. This is just not possible without someone repeating Dr. Liley's classic experiments.

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Reply

To the Editors:

I agree with the comments of Dr. Duhring. The points are repeatedly stressed in the paper that reviewed the methodology used by Dr. Liley and stresses that in the third trimester amniocentesis and interpretation of delta optical density at 450 nm values by the Liley method have proved to be reliable. However, the findings of the paper suggest that in the second trimester of pregnancy, fetal blood sampling is the only accurate method of assessing the severity of fetal anemia.

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Hypertensive diseases of pregnancy: Nulliparity, primiparity, multiparity, and gravidity

To the Editors:

In an otherwise fine article (Hypertensive diseases of pregnancy and parity. Am J Obstet Gynecol 1986; 154:1044-9) on hypertensive diseases of pregnancy and parity, Gleicher et al.,[1] while criticizing the vague and "somewhat" arbitrary terminology of hypertensive diseases, fail to accurately designate their two subsets of patients, presumably those who have not been delivered of a fetus or fetuses who have reached the stage of viability and those who have. If parity is the variable in question, then the two groups of gravidas who are compared are nulliparous and parous women and *not* primiparous and multiparous women. Likewise, nulliparous and *not* primiparous patients make up the bulk of patients with "true" preeclampsia. Parous patients include those who are para 1 or primiparas and para 2 and greater or multiparas.

Further confusion about the terminology is suggested in the introduction where primigravid women (excluding multigravid nulliparas) are compared with multiparas, the proverbial apples and oranges dichotomy. Finally, after parity is described as the distinctive study variable, the authors conclude that "*gravidity* is the single best option in the differential diagnosis of hypertensive diseases during pregnancy."

Is *parity* the significant variable?

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REFERENCE

1. Pritchard JA, MacDonald PC, Gant NF. Williams' obstetrics. Norwalk, Connecticut: Appleton-Century-Crofts, 1985:246.

Reply

To the Editors:

We appreciate the interest in our paper but have to disagree with Reamy's well-meant criticism of our terminology. Patients included in this study were already delivered when their status was analyzed. In fact, weight determinations of newborn infants probably represented the most important outcome variable of this study. To classify women who had given birth to their first child as "primiparous" and others as "multiparous" consequently appears correct and understandable to the reader.

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Pemphigus in pregnancy: A reevaluation of fetal risk

To the Editors:

We are much impressed with the paper by Ross et al. (Pemphigus in pregnancy: A reevaluation of fetal risk. Am J Obstet Gynecol 1986;155:130-3) on fetal risk in pregnancy with pemphigus vulgaris. Our experience with a live baby affected with neonatal pemphigus prompted us to review the pertinent literature.[1]

Not surprisingly, we concur with Ross et al. regarding their conclusion that the fetus of a mother with pemphigus is at risk of intrauterine death (23% stillbirth rate). Their considerations were also raised by us, namely, that high dose of corticosteroids either causes placenta insufficiency or encourages overwhelming infection in the fetus and thus intrauterine death. We also believe that it is unlikely that pemphigus vulgaris is the cause of stillbirth.

One way to curb fetal risk in cases of pemphigus vulgaris in the pregnant woman can be the repeated use of plasmapheresis. This should be considered when the woman in question either: (1) requires a high dose of corticosteroids, (2) suffers from any opportunistic infection, (3) has a high titer of circulating pemphigus antibodies, or (4) when the fetal well-being profile (biophysical and biochemical) is low.[2] Plasmapheresis was suggested in the past for cases of pemphigus vulgaris,[3] but not advocated for pemphigus during pregnancy. This might be a useful measure until the completion of pregnancy instead of high-dose corticosteroid treatment and might presumably decrease the fetal risk in these precarious cases.

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REFERENCES

1. Merlob P, Metzker A, Hazaz B, Rogovin H, Reisner SH. Neonatal pemphigus vulgaris. Pediatrics 1986;78:11: 1102-5.
2. Polin JI, Frangipaut WL. Current concepts in management of obstetric problems for pediatricians. I. Monitoring the high risk fetus. Pediatr Clin North Am 1986;33:621-47.
3. Ruocco V, Rossi A, Argenziano G, et al. Pathogenicity of the intercellular antibodies of pemphigus and their periodic removal from the circulation by plasmapheresis. Br J Dermatol 1978;98:237-41.

The cause of uterine softening in pregnancy

To the Editors:

It was with great interest that we read the article of Dr. Munsick (Dickinson's sign: Focal uterine softening in early pregnancy and its correlation with the placental site. AM J OBSTET GYNECOL 1985;152:799-802) with regard to his experience about the site of implantation of the placenta as observed during artificial terminations of the pregnancy. In his experience, the site of softening corresponded with that of the implantation of the placenta in 88% of the cases.

Our pertinent experience rests in 150 observations based on cases of normal pregnancy between the sixth and twentieth weeks of gestation. When the softening was widespread, we attempted to establish the site of maximum softening. We found that the softening was most frequent in the fundal area of the uterus (Table I).

In 68 cases we used real-time ultrasound examination for the determination of placental localization. In this regard, our findings are similar to those of Dr. Munsick: The sites of implantation and softening were identical in 54 instances (79.42%) and differed in six cases (8.82%). In the remaining eight cases (11.76%), we could not identify a specific area of softening. Of the latter cases, the site of implantation was the posterior uterine wall in seven patients in whom it appears that it is more difficult to appreciate the occurrence of uterine softening than elsewhere. In one instance we found the uterus rather contractile, a circumstance that hindered the accuracy of the observation.

Table I. Distribution of the sites of uterine softening in pregnancy

Site of softening	*No. of cases*	*%*
Fundus	40	26.67
Right uterine wall	36	24.00
Left uterine wall	31	20.67
Anterior uterine wall	11	7.33
Posterior uterine wall	6	4.00
Undecided	26	17.33
Total	150	100.00

The fact that in approximately 80% of our cases the site of softening coincided with that of placental implantation, appears to support the concept of Csapo and Pulkinen[1] concerning the differing consistency of the myometrium in areas adjacent to or remote from the site of placental implantation, a phenomenon leading to functional asymmetry.

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REFERENCE

1. Csapo AI, Pulkinen MO. Regulatory and functional asymmetry of the pregnant uterus. Perinatol Neonatol 1977; 1:12.

Intrapartum electronic fetal monitoring efficacy

To the Editors:

On the basis of a review of seven randomized controlled clinical trials, Dr. Thacker concludes in his report (The efficacy of intrapartum electronic fetal monitoring. AM J OBSTET GYNECOL 1987;156:24) that electronic fetal monitoring is not a useful procedure for all women in labor. He states "while the randomized controlled clinical trial is the most powerful tool available to assess a clinical practice such as electronic fetal monitoring, even this tool has limitations."

In at least one report cited by Dr. Thacker, electronic fetal monitoring was used as a screening test to determine which patients were high risk and needed electronic fetal monitoring.

However, what is most significant are the two reports not discussed by Dr. Thacker. In the report by Ingemarrson et al., "Impact of routine fetal monitoring during labor on fetal outcome with long-term follow-up (AM J OBSTET GYNECOL 1981;141:29), the authors concluded, "The improved short-term and long-term fetal outcomes seem to be largely a result of routine fetal monitoring with all its implications for obstetric and neonatal management; the significant reduction in neurological sequelae, even after correction for other

changes in obstetric routines supports this suggestion." In the report by Erkkola et al., "Analysis of intrapartum fetal deaths: their decline with increasing electronic fetal monitoring" (Acta Obstet Gynecol Scand 1984;63: 459) the authors concluded that when 95% of all vaginal deliveries received electronic fetal monitoring in the fourth consecutive 3-year period of the study, intrapartum fetal deaths (IFDs) were 0.3/1000 compared with 1.7/1000 in the first 3-year period of the study when only 9% of labors were monitored electronically. The authors add, "our material gives strong support to the numerical speculation of Quilligan and Paul that in an obstetric service with 3000 deliveries annually, by undertaking 100% monitoring, 6 IFDs might be avoided."

As a practicing clinician caring for the life, health, and welfare of mothers and their fetuses in the labor and delivery suite and not at a distance in an office chair, I still remember the occasions, fortunately few, of being called by the labor room nurse to come attend what turned out to be an intrapartum fetal death in a mother not receiving the benefit of continuous electronic fetal monitoring in labor, whereas I have never experienced to date an intrapartum fetal death in a mother who was the beneficiary of continuous electronic fetal monitoring in labor. Therefore, on the basis of reported studies and clinical experience I conclude that the benefits of electronic fetal monitoring in all labors far outweigh either the risks of electronic fetal monitoring and/or not using electronic fetal monitoring, as seems proved so far at least for the prevention of intrapartum fetal deaths and fetal neurologic sequelae. The prevention of these two dire conditions, one fatal and the other disabling, is enough for me to justify that the beneficial cost-efficiency of routine electronic fetal monitoring in labor adds up in the black on the financial balance sheet and in the physical and emotional health and welfare of the specific persons involved and society in general.

This is a personal communication of the author and is in no way to be construed as representing either Kaiser Permanente or Southern California Permanente Medical Group.

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Reply

To the Editors:

In my paper on the efficacy of electronic fetal monitoring, I assessed the effectiveness of electronic fetal monitoring as an intrapartum screening tool.[1] I presented a systematic method for evaluating the evidence from seven randomized controlled clinical trials and concluded that benefits might be limited to the reduction of neonatal seizures and that the data did not demonstrate that electronic fetal monitoring is a useful screening procedure for all women in labor. Dr. Fribourg is a concerned clinician who believes that the routine use of electronic fetal monitoring is important to "the health and welfare of mothers and their fetus[es]." To support his contention, he cites his clinical experience and two reports from the clinical literature.

The approach in my report was based on epidemiologic evidence. Because efficacy of a clinical procedure can best be measured in randomized controlled clinical trials, I focused on the data from randomized controlled clinical trials to assess the effectiveness and safety of routine electronic fetal monitoring. The two reports Dr. Fribourg cited are not randomized controlled clinical trials and for that reason were not included in my analysis. Taken together, the seven randomized controlled clinical trials do not demonstrate a significant decrease in intrapartum deaths atributable to routine electronic fetal monitoring. As indicated in my paper, the Dublin trial[2] shows that routine electronic fetal monitoring is associated with a significant decrease in neonatal seizures but not a decrease in serious neurologic sequelae. Two subsequently published reports on clinical trials designed to assess electronic fetal monitoring support my conclusions.[3, 4]

As a medical epidemiologist, I use epidemiology as a tool to address problems that routinely confront clinicians such as Dr. Fribourg. Since many of our health care practices are being assessed with epidemiologic studies, it is important that such studies be critically reviewed. Clinicians can then interpret and use this information on the basis of their experience in patient care. Hopefully, by combining epidemiologic evidence and clinical understanding, we can maximize women's and infants' health.

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REFERENCES

1. Thacker SB. The efficacy of intrapartum electronic fetal monitoring. Am J Obstet Gynecol 1987;156:24-30.
2. MacDonald D, Grant A, Sheriden-Pereira M, et al. The Dublin randomized control trial of intrapartum fetal heart rate monitoring. Am J Obstet Gynecol 1985;152:524-39.
3. Leveno KJ, Cunningham FG, Nelson S, et al. A prospective comparison of selective and universal electronic fetal monitoring in 34,995 pregnancies. N Engl J Med 1986; 315:615- 9.
4. Luthy DA, Shy KK, van Belle G, et al. A randomized trial of electronic fetal monitoring in preterm labor. Obstet Gynecol 1987;69:687-95.

Previous cesarean delivery and trial of labor

To the Editors:

The basic premise stated in the first paragraph of the article by Finley and Gibbs (Emergent cesarean delivery in patients undergoing a trial of labor with a

transverse lower segment scar. AM J OBSTET GYNECOL 1986;155:936), "that a trial of labor be allowed only in labor and delivery units with continuous fetal monitoring, 24-hour blood banking, and an operative start-up time of 30 minutes implies an increased intrapartum risk," is wrong. The aforementioned recommendations are taken from a February 1985 publication from the Committee on Obstetrics: Maternal and Fetal Medicine of the American College of Obstetricians and Gynecologists.

However, *Standards for Obstetric-Gynecologic Services* (6th ed. Washington, DC: The American College of Obstetricians and Gynecologists, 1985:29-30) sent to Fellows of the College in June 1985 states, "Any facility providing obstetric care, however, should have the following minimum services available," and then proceeds to itemize 11 requirements, which include all those mentioned by Drs. Finley and Gibbs.

Therefore it seems that in fact patients with a previous cesarean undergoing a trial of labor are considered to be at the same risk as those patients in labor without a previous cesarean, which is contrary to what the authors have written, and that the same minimal level of obstetric care is required by (or *recommended for*) all patients.

This is a personal communication of its author and is in no way is to be construed as representing either Kaiser Permanente or Southern California Permanente Medical Group.

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Reply

To the Editors:

We appreciate the comments and criticisms provided by Dr. Fribourg. The intent of our initial paragraph was to restate the obvious—preconceived risks are associated with a trial of labor in the minds of many clinicians. The existence of such preconceptions is made obvious by the current low frequency of trial of labor in patients with a prior low transverse cesarean section (8%) and by the fact that only 46% of hospitals allow trial of labor.[1] We did not, however, mean to imply that the Committee on Obstetrics: Maternal/Fetal Medicine or the American College of Obstetrics and Gynecology attaches such increased risks to a trial of labor. The existence of such communication by the Committee on Obstetrics: Maternal/Fetal Medicine, however, demonstrates a response to such preconceived risks.

As described in our second paragraph, it was our intent to address the hazards associated with a trial of labor in a defined clinical setting. We believe that our data concur with the flavor or Dr. Fribourg's letter—a trial of labor in selected patients does not place them at undo risk. It is our hope that such data will allow for an increase in the percentage of appropriate candidates being proffered a trial of labor in the future.

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REFERENCE

1. Schiono PH, Fielden JG, McNellis D, Rhoads GG, Pearse WH. Recent trends in cesarean birth and trial of labor rates in the United States. JAMA 1987;257:494.

HELLP syndrome

To the Editors:

In the report by Sibai et al. (Maternal-perinatal outcome associated with the syndrome of hemolysis, elevated liver enzymes, and low platelets in severe preeclampisa-eclampsia. AM J OBSTET GYNECOL 1986; 155:501-9), the prognosis with a "true" HELLP syndrome in preeclampsia was associated with a poor maternal-perinatal outcome.

What is confusing is Dr. Sibai's conclusion that he could work with these mothers using the steroids and wait 48 to 72 hours. This is a catastrophic dilemma. Even in his 1985 article he stated that there was no justification for conservative management of severe preeclampsia.

To improve gestational age we delay the delivery of babies who are sick. The babies have a higher incidence of growth retardation and a higher incidence of low Apgar scores. The steroid therapy did not improve the pregnancy outcome.

Once the diagnosis of severe preeclampsia is made, the only therapy would be to get the patient delivered. This was Dieckmann's dictum in the early 1950s, and it is true today.

With the aid of Dr. Weinstein's HELLP syndrome and the use of serial platelet counts, we can arrive at an earlier diagnosis and the degree severity of the disease process.

Our conclusion would differ from that of Dr. Sibai in that steroid therapy has no place in the treatment of severe preeclampsia. The time delay is more detrimental to the mother than the benefits of increase in gestational age.

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REFERENCES

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2 Garite TJ, Freeman RK, Linzey EM, Braly PS, Corchester WL. Prospective randomized study of corticosteroids in the management of premature rupture of the membranes and the premature gestation. AM J OBSTET GYNECOL 1981;141:508-16.
3. Weinstein L. Syndrome of hemolysis, elevated liver enzymes, and low platelet counts: a severe consequence of hypertension in pregnancy. AM J OBSTET GYNECOL 1982;142:159-67.

4. Sibai BM, Taslimi M, Abdella TN, et al. Maternal and perinatal outcome of conservative management of severe preeclampsia in midtrimester. AM J OBSTET GYNECOL 1985;152:32-7.

Reply

To the Editors:

I would like to thank Dr. Gerace for his interest in our article.

Dr. Gerace questioned the value of using steroids in patients with severe preeclampsia, and he recommended immediate delivery of all such patients, irrespective of gestational age. He cited some references (Dieckmann and Weinstein), and yet he supplied none of his own data. The data regarding the benefits from steroids in preeclampsia are confusing, but a benefit for such therapy has been reported by several authors (see references in our article). Our data did not demonstrate such a benefit in our population. Hence, the recommendation in the closing remarks of the paper was for patients with minimal laboratory findings (not true HELLP) as reported by others. The benefits of using steroids in preeclampsia will be the subject of an upcoming report from this institution. (Gonzalez and Sibai. In preparation.)

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Books received

Atkinson's Correlative Atlas of Colposcopy, Cytology, and Histopathology. Robert L. Giuntoli, Barbara F. Atkinson, Carolyn S. Ernst, Mary M. Rubin, and Virginia S. Egan. 288 pages, illustrated. Philadelphia, 1987, J. B. Lippincott Company.

Chlamydial Infections. Edited by David Oriel, Geoff Ridgway, Julius Schachter, David Taylor-Robinson, and Michael Ward. 583 pages, illustrated. New Rochelle, New York, 1986, Cambridge University Press. No price listed.

Compendio di Isterosalpingografia. A. Tetti and C. Tetti. 92 pages, illustrated. Torino, Italy, 1987, Edizioni Minerva Medica.

Complications in the Management of Breast Disease. Edited by R. W. Blamey. 272 pages, illustrated. Philadelphia, 1987, W. B. Saunders Company. $38.95.

Endometriosis. Daniel T. O'Connor. 154 pages, illustrated. New York, 1987, Churchill Livingstone Inc. $33.00.

The Experience of Breastfeeding. Sheila Kitzinger. 239 pages. Middlesex, England, 1987, Penguin Books Ltd. $6.95 (soft cover).

Future Aspects in Human In Vitro Fertilization. W. Feichtinger and P. Kemeter. 285 pages, illustrated. Berlin, 1987, Springer-Verlag. $51.00.

A Handbook of Obstetrics & Gynaecology: A Guise for Housemen. R. S. Ledward. 248 pages, illustrated. Littleton, Massachusetts, 1986, PSG Publishing Company. $15.00 (soft cover).

How to Persuade Your Lover to Use a Condom and Why You Should. Patti Breitman, Kim Knutson, and Paul Reed. 83 pages. Rocklin, California, 1987, Prima Publishing and Communications. $4.95 (soft cover).

Listening to Gynaecological Patients' Problems. David Jenkins. 104 pages. Berlin, 1986, Springer-Verlag. No price listed (soft cover).

New Developments in Imaging. Edited by Rainer Ch. Otto and Charles B. Higgins. 172 pages, illustrated. New York, 1986, Thieme Medical Publishers. $47.00.

Obstetric Ultrasound: How, Why, and When. Patricia Chudleigh and J. Malcolm Pearce. 194 pages, illustrated. New York, 1986, Churchill Livingstone Inc. $28.00.

Physiologic Foundations of Perinatal Care. Volume 2. Leo Stern, William Oh, and Bent Friis-Hansen. 420 pages, illustrated. New York, 1987, Elsevier Science Publishing Co., Inc. $63.00.

PMS—A Positive Program to Gain Control. Stephanie DeGraff Bender and Kathleen Kelleher. 245 pages. Tucson, 1986, The Body Press. $7.95.

Problems in Obstetric Anaesthesia. Barbara Morgan, Guest Editor. 198 pages. New York, 1987, John Wiley & Sons. No price listed.

Progress in Obstetrics and Gynaecology. Volume 6. Edited by John Studd. 442 pages, illustrated. New York, 1987, Churchill Livingstone Inc. $36.95.

Reproduction in Mammals: Manipulating Reproduction. C. R. Austin and R. V. Short. 235 pages, illustrated. London, 1986, Cambridge University Press. $15.95 (soft cover).

Synopsis of Gynecologic Oncology. Third Edition. C. Paul Morrow and Duane E. Townsend. 584 pages, illustrated. New York, 1987, John Wiley & Sons. $45.00.

Ultrasound Diagnosis in Obstetrics and Gynecology. M. Hansmann, B. J. Hackeloer, and A. Staudach, with B. K. Wittmann, co-editor. 495 pages, illustrated. Berlin, 1986, Springer-Verlag. $100.00.

When Parents Die: A Guide for Adults. Edward Myers. 208 pages. New York, 1986, Penguin Books. $6.95 (soft cover).

ITEMS

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The First National Fragile X Conference, December 3 and 4, 1987, Denver, Colorado. For information contact: The Fragile X Foundation National Conference, c/o Expectations Unlimited, Inc., 6897 Paiute Ave., #2, Longmont, CO 80501.

Understanding AIDS, December 11-12, 1987, Red Lion Inn, Omaha, Nebraska. Sponsored by the Division of Maternal-Fetal Medicine and the Division of Infectious Disease, Creighton University School of Medicine, Department of Obstetrics and Gynecology. For information contact: Hattie DeLapp, Creighton University School of Medicine, Omaha, NE 68178. Tel.: 1-800-228-7212, ext. 2550; in Nebraska: 1-402-280-2550.

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The Fort Collins Women's Clinic, a busy four-person OB/GYN Group in Fort Colins, CO is looking for an industrious, progressive obstetrician-gynecologist to join them in practice.

The Women's Clinic offers an excellent opportunity leading to eventual partnership along with an excellent benefit package, including malpractic insurance.

Fort Collins, located 60 miles north of Denver, offers a delightful, suburban, family oriented environment.

Please send CV. All replies confidential.

Address:

Practice Administrator
Fort Collins Women's Clinic
1120 East Elizabeth, Bldg. F
Fort Collins, CO 80524

PACIFIC NORTHWEST

Expanding Practice Seeks OB/Gyn

Ground floor opportunity with BC/BE women.

- Diverse urban clientele.
- Affiliation with major hospital.
- First year income guarantee.
- New family-oriented delivery facility.
- Malpractice, other benefits.
- Partnership potential.

For detailed fact sheet, contact:

THE FRIEDRICH GROUP
9284 Ferncliff, N.E.
Bainbridge Island, WA 98110
(206) 547-7850 or (206) 842-5248

OB/GYN

Excellent opportunities available for a Board Eligible or Certified OB/GYN Physician in the beautiful Finger Lakes area of New York State. The area is served by a modern well equipped Hospital - 265 beds. Unusual benefits available. Inquiries may be addressed to:

C.D. Hicks
Physician Procurement Committee
157 Ross Street
Auburn, New York 13021
or call
(315) 253-3388

JOHNS HOPKINS SCHOOL OF HYGIENE AND PUBLIC HEALTH

The Department of Maternal and Child Health, The Johns Hopkins University, is seeking a full-time faculty member to strengthen its teaching and research in the area of maternal health. Applicants must have a doctoral degree with training in either epidemiology or health services research. Evidence of research productivity is required. Preference will be given to individuals with experience in applied epidemiology or health services research and with a professional background in medicine, nursing or nutrition. Faculty rank and salary are negotiable. Send CV and letter explaining interests to Chairman, Department of Maternal and Child Health, 615 N. Wolfe St., Baltimore, MD 21205. Equal Opportunity Employer (M/F/H).

OBSTETRICIAN/ GYNECOLOGIST

Outstanding opportunity for experienced BC/BE OB-GYN physician to associate with Kaiser Permanente's Kansas City Region. Academically affiliated program with housestaff support, competitive salary and benefit structure including malpractice coverage, family oriented metropolitan area.
Send CV to:

Larry McDonald, M.D.
4240 Blue Ridge Blvd. Suite 1000
Kansas City, MO 64133

816/737-2800

OB/GYN

WANTED: Obstetrician/Gynecologist, Board Certified or eligible physician to join existing 3 member OB/GYN Department in 22 member multi-specialty group practice in the Lakes Region of central N.H. Position offers an outstanding professional, financial, and life-style opportunity. Respond with C.V. to Alan Awrich, M.D., Laconia Clinic, 724 Main Street, Laconia, NH 03246.

PERINATOLOGISTS

Illinois, Chicago - Lutheran General Hospital is seeking two Perinatologists for an excellent opportunity in direct patient care, teaching and clinical research. Lutheran General is a 700-bed teaching hospital affiliated with the University of Illinois College of Medicine in Chicago. It is a designated tertiary care center with 3600 deliveries annually and has an active maternal transport service of over 200 a year. The Obstetrics and Gynecology Department has a residency program, and subspecialists in Gynecology/Endocrinology and Gynecology/Oncology. There is a 36-bed neonatal NICU with five neonatologists and a fellowship program. There is also a large referral service for genetic counseling and prenatal diagnoses, and more than 750 genetic amniocentesis are preformed annually. We have recently opened a large Perinatal Tertiary Center office.

The hospital is located in an attractive northwest suburb of Chicago. We offer a competitive salary with a generous incentive plan and an excellent benefit package.

Please send your curriculum vitae to:

Dr. Haim Elrad
Director of Maternal-Fetal Medicine
Division of Obstetrics and Gynecology

Lutheran General Hospital
1775 Dempster Street
Park Ridge, Illinois 60068
or call (312) 696-6998

REPRODUCTIVE ENDOCRINOLOGIST

Marshfield Clinic, a 250 physician multispecialty private group practice in Central Wisconsin is seeking a Fellow trained BC/BE Reproductive Endocrinologist to join its 11 member Department of OB/GYN which includes a Perinatologist and Gynecologic Oncologist. The Clinic serves as a major regional referral center and provides a comprehensive spectrum of existing supporting specialties and services. An adjacent 524 bed acute care teaching hospital includes a Level III Perinatal Center and a 24 bed Neonatal Unit. Teaching and research opportunities also exist. Please send curriculum vitae and the names of several references to:

Mr. John P. Folz
Director-Patient Services
1000 North Oak Avenue
Marshfield, WI 54449
or call collect at
(715) 387-5181

Marshfield Clinic

OBSTETRICIAN GYNECOLOGIST-IN-CHIEF

The Mary Imogene Bassett Hospital, a 189-bed, rural referral center, and major teaching affiliate of Columbia University, is seeking candidates for this key appointment. The chief is responsible for overall clinical and teaching programs in Obstetrics and Gynecology. The Service is providing approximately 500 deliveries and about 4300 obstetrical clinic visits yearly. Volume in Gynecology is about 290 admissions and 7800 clinic visits. The department has four attending physicians, rotating transitional residents, a certified nurse midwife, a physician's assistant, plus substantial nursing and office staffing.

Cooperstown, on scenic Lake Otsego about 60 miles west of Albany, enjoys unusual recreational opportunities, a strong school system, and an array of cultural and artistic activities. The village is known for its air of history, museums and resort facilities.

The medical staff is organized as a salaried group practice with full fringe benefits including malpractice coverage, generous free time, conference leave, and immediate annuity participation.

Please send curriculum vitae and correspondence to:

Harlan D. Alpern, M.D.
Search Committee Chairman
The Mary Imogene Bassett Hospital
Cooperstown, New York 13326
telephone (607) 547-3700

THE MARY IMOGENE
BASSETT HOSPITAL

OB/GYN

Fifth MD needed summer of 1987 to join department of 4 MD's, 2 CNM's and 1 NP in a multispecialty group practice in Nashua, NH one hour from Boston and seacoast. Salary range of $70-116,000 plus excellent benefits and malpractice insurance. Applicants must be board certified or eligible. Please contact:

Karen Reed
Matthew Thornton Health Plan
CS#2028
Nashua, NH 03061
(603-883-2800)

Faculty Position
GEORGIA

Chief, OB/GYN in Community Hospital. Academic setting teaching Family Practice Residents who staff busy OB/GYN service in tertiary perinatal center. Emory University and Medical College of Georgia affiliate. Relaxed professional environment.

Send C.V. to:

Howard G. Vigrass, M.D.
710 Center Street
Box #84
Columbus, GA 31994

MATERNAL-FETAL MEDICINE

The Department of Obstetrics and Gynecology, Medical University of South Carolina, is accepting applications for a full-time faculty position in an established Section on Maternal-Fetal Medicine. Applicants must be Board eligible or certified in MFM. Duties includes patient care, instruction of medical students and residents, and participation in perinatal research. Academic appointment and salary dependent on previous experience.

Interested candidates should send CV to:

Edgar O. Horger III, M. D.
Department of Obstetrics and Gynecology
Medical University of South Carolina
171 Ashley Avenue
Charleston, South Carolina 29425

Michael Reese Health Plan

The **Michael Reese Health Plan**, a large, well-established HMO serving its members in 14 Health Centers throughout the Chicago Metro area, due to growth, is seeking additional Board Certified/Eligible physicians in the following specialty:

Obstetrics
Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching medical students and house staff.

A highly-competitive salary and excellent benefits compliment a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Stuart P. Bowne, M.D., Medical Director
MICHAEL REESE HEALTH PLAN
2545 King Drive, Chicago 60616

or call (312) 808-3810

an equal opportunity employer m/f/h

TWO POSITIONS AVAILABLE:

FULL TIME GYN ONCOLOGIST AND OBSTETRICIAN/GYNECOLOGIST DEPARTMENT OF OB/GYN MOUNT SIANI MEDICAL CENTER OF GREATER MIAMI

Excellent opportunity fo BE/BC GYO. Hospital-based position affiliated with the University of Miami.

Opportunity for research and teaching. Positions immediately available. Must be eligible for or have Florida license.

If interested, please send Curriculum Vitae or contact:

Arthur G. Shapiro, M.D.
Chairman, Department of Ob/Gyn
Mount Sinai Medical Center
4300 Alton Road
Miami Beach, Florida 33140
Telephone No. (305) 674-2833

THE MOUNTAINS ARE BEAUTIFUL AND WEATHER COOL.

OB-GYN—If you are a board certified or finishing resident who might be interested in a rewarding professional practice, give Murphy, N.C. serious consideration. Murphy Medical Center is a modern, seven year old, 50 acute care and 120 long term care bed facility which serves a four county population of 45,000+. Located in the foothills of the Smokey Mountains, Murphy offers the advantages of a small-town lifestyle, with numerous outdoor activities, without the sacrifice of urban advantages. Atlanta or Asheville are under a two hours drive from Murphy. Attractive first year financial guarantee is available. For more information write or call:

CEO, Murphy Medical Center
2002 U.S. Highway 64 East
Murphy, N.C. 28906
(704) 837-8161
E.O.E.

FACULTY POSITIONS OBSTETRICS & GYNECOLOGY

The Orlando Regional Medical Center, Orlando, Florida is seeking full-time faculty, including Perinatologist, for its OB/GYN Residency Program. Please send CV and three letters of reference to:

Orlando Regional Medical Center
Vice President/Medical Education
1414 S. Kuhl Avenue
Orlando, FL 32806

OB/GYN GENERALIST

Pennsylvania Hospital seeks 2 board certified or eligible obstetrician/gynecologists. One position is available as of January 1, 1988, the other as of July 1, 1988. Responsibilities include resident and medical student education and active clinical practice. Appointment is at the assistant professor level. Applications from women and minority persons are especially welcome.

Pennsylvania Hospital is an affiliate of the University of Pennsylvania and is an affirmative action/equal opportunity employer.

Interested candidates should send a curriculum vitae and letter to:

Linda K. Dunn, M.D.
Department of Obstetrics and Gynecology
Pennsylvania Hospital
8th & Spruce Streets
Philadelphia, PA 19107

KAISER PERMANENTE

Good People. Good Medicine.

Maternal-Fetal Medicine

BC/BE Perinatologist wanted to join another subspecialist in a large multispecialty HMO located in the San Francisco-Oakland Bay Area.

Responsibilities include clinical service in two tertiary referral centers with 6,000 deliveries/year; consultations in high risk patients, genetic testing, ultrasound, resident teaching, and perinatal outreach programs. CVS experience desirable.

Each institution with full NICU capabilities with total of six neonatologists. Oakland facility with approved residency program (12 residents), oncologist, endocrine-infertility subspecialists, Level II Ultrasound capabilities and antenatal testing unit.

Excellent benefits including competitive salary, malpractice insurance, medical and dental coverage, vacation and sick leave, pension and retirement programs. University affiliation and clinical research available.

Please write or call, including your curriculum vitae and three references:

Victor Chin, M.D., FACOG
Chief, Department of Obstetrics & Gynecology
The Permanente Medical Group, Inc.
280 West MacArthur Blvd.
Oakland, CA 94611
415 428-7340
or Lawrence Newman, M.D., FACOG
Perinatologist
415 428-7087

OBSTETRICIAN GYNECOLOGIST

Board eligible/board certified obstetrician/gynecologist wanted to join expanding Ob/Gyn Department of Southboro Medical Group, Inc. Southboro Medical Group is a multispecialty group practice located 30 minutes of Boston in the affluent town of Southboro, Massachusetts. There are currently two full time board certified ob/gyn's in the department who are very busy and hope to eventually have two additional ob/gyn's to share the practice. This position offers a generous salary, the potential for incentive bonus during the first two years, and excellent fringe benefits including paid malpractice insurance. Please contact:



Michael S. Robertson, M.D.
Southboro Medical Group, Inc.
P.O. Box E1
Southboro, MA 01772.

Obstetricians/ Gynecologists

St. Margaret's Hospital for Women is seeking several Board Certified/Board Eligible Obstetricians/Gynecologists to join our expanding group private practice in affiliation with the Hospital. St. Margaret's is one of the largest Catholic specialty hospitals in OB/GYN, and a major perinatal and neonatal center, and is affiliated with Tufts University School of Medicine.

Excellent salary and benefits are offered. Interested candidates should respond with curriculum vitae to: Linda Caliga, President, St. Margaret's Hospital for Women, 90 Cushing Ave., Boston, MA 02125.

An equal opportunity employer employer

ST. MARGARET'S HOSPITAL FOR WOMEN

PERINATOLOGISTS

Three full-time positions are available as perinatologists in Maternal-Fetal Medicine in the Department of OB/GYN, U.C. Davis Medical Center, Sacramento. Applicants should be Board eligible or certified in OB/GYN and must have successfully completed subspecilaty training in Maternal-Fetal Medicine with subsequent Specialty Board eligibility and/or certification. Applicants must demonstrate experience and competence in teaching effectiveness and research related activities. Applicants must demonstrate experience in prenatal diagnosis, ultrasound and genetics.

Responsibilities include inpatient and outpatient case management, surgery and procedures specific to high risk obstetrics, participation in teaching activities with residents, interns and medical students, development and/or participation in research activities and involvement in University and Hospital committees.

Send curriculum vitae and five references to:

R. Jeffrey Chang, M.D.
Chairman, Department of Obstetrics and Gynecology
University of California, Davis, Medical Center
1621 Alhambra Boulevard, Suite 2500
Sacramento, California 95816

The final date for receipt of appplications is "Open until filled, but not later than April 1, 1988"

The University of California, Davis is an equal opportunity, affirmative actior employer.

FACULTY POSITION

Department of Obstetrics and Gynecology
University of Massachusetts Medical Center

We are seeking board certified Obstetricians/Gynecologists to join our University Group of Obstetricians. Full time faculty positions offer excellent opportunities in resident education, active clinical practice and research.

Academic level will be in accordance with experience and achievement. Along with competitive salary, benefits include; membership in a multi-specialty Group Practice Plan, paid professional liability insurance, state and group retirement plans, health, dental, life, and disability insurance.

Located in Central Massachusetts, the area provides year round recreational opportunities, a variety of residential styles and abundant educational/cultural options.

Interested candidates should send a curriculum vitae and letter to:

Richard E. Hunter, M.D.
Chairman, Ob/Gyn
University of Mass. Medical Center
55 Lake Avenue North
Worcester, MA 01655

GYNECOLOGIC ONCOLOGIST
DIVISION OF GYNECOLOGIC ONCOLOGY
UNIVERSITY OF WASHINGTON

Full time position available for a Board certified or Board eligible gynecologic oncologist to be based in a large community hospital near Seattle in a program affiliated with the University of Washington School of Medicine. Full academic appointment commensurate with experience. Must be eligible for or have Washington state licence.

Send Curriculum Vitae or CONTACT:

David C. Figge, M.D.
Director, Gynecologic Oncology
University Hospital (RH-20)
University of Washington
Seattle, WA 98195

AN EQUAL OPPORTUNITY EMPLOYER

OBSTETRICS AND GYNECOLOGY

The Department of Obstetrics and Gynecology at the **University of Wisconsin/Madison** is seeking 2 faculty members, 1 for general Obstetrics/Gynecology and 1 for the General Gynecology Service.

The General Obstitrician/Gynecologist will act as consultant to a staff-model HMO. For the faculty member position, colposcopy and laser experience are highly desirable. Both positions involve supervision and teaching of residents and students, the general practice of Obstetrics and Gynecology within the departmental practice plan, and some administration. Opportunity for clinical research available. Academic rank and salary commensurate with experience. Inquiries to: **C.B. Martin, Jr., MD, Department of Obstetrics & Gynecology, H4/654, Clinical Science Center, 600 Highland Avenue, Madison, WI 53792.**

University of Wisconsin Hospital & Clinics

The University of Wisconsin is an Equal Opportunity/Affirmative Action Employer.

Faculty Position in Maternal-Fetal Medicine

The Department of Obstetrics and Gynecology at Wright State University School of Medicine invites applications for a full-time faculty position in Maternal-Fetal Medicine combined with the position of Medical Director of a regional perinatal care center.

Primary duties will include the teaching of medical students and residents, the pursuit of scholarly activities, and the medical administration of the perinatal center which includes the obstetrical unit and the labor and delivery suite.

Applicant qualifications include the M.D. degree, board certification in obstetrics and gynecology, licensability to practice Medicine in Ohio, completion of a fellowship in maternal-fetal medicine, board eligibility or certification in maternal-fetal medicine, and extensive training and experience in perinatal medicine and administration of health services. Academic rank and salary will be commensurate with qualifications and professional experience. Please send curriculum vitae and the names of three references to: John J. Halki, M.D., Ph.D., Chair and Program Director, Department of Obstetrics and Gynecology, Wright State University School of Medicine, 1 Wyoming Street, Dayton, OH 45409.

The Wright State University School of Medicine is an Affirmative Action and Equal Opportunity Employer.

All applications received by November 30, 1987 will be considered; if the position is not filled therefrom, applications will be considered as received until the position is filled.

POSITION AVAILABLE

Our medical group is actively engaged in the practice of gynecology and obstetrics in the metropolitan Detroit, Michigan area. As a result of growth, we are prepared to add one additional associate.

This opportunity provides compensation and fringe benefits considerably greater than you might otherwise expect. In addition, permanent participation in our group is anticipated.

If you are interested in joining our group, we would be pleased to discuss these matters with you in greater detail. Your inquiry will be kept in confidence if you so desire. Please call collect or direct replies to:

Roger M. Kushner, D.O.
27301 Five Mile Road
Redford, MI 48239
Telephone: (313) 535-8484

INDEX TO ADVERTISERS

Air Force Hospital
Opportunity Available 51A

Ayerst Laboratories
Premarin 16A,17A,18A

Berlex Laboratories, Inc.
Levlen 21A,22A
Tri-Levlen 48A,49A,50A

Carle Clinic
Opportunity Available 51A

Ciba Pharmaceutical Company
Estraderm 43A,44A,45A,46A

Fort Collins Women's Clinic
Opportunity Available 51A

Friedrich Group, The
Opportunity Available 47A,51A

Hicks, C. D.
Opportunity Available 52A

John Hopkins School of Hygiene and Public Health
Opportunity Available 52A

Kaiser Permanente
Opportunity Available 47A

Kaiser Permanente, Kansas City
Opportunity Available 52A

Kushner, Roger, M.
Opportunity Available 57A

Laconia Clinic
Opportunity Available 52A

Lutheran General Hospital
Opportunity Available 53A

Marshfield Clinic
Opportunity Available 47A,53A

Mary Imogene Bassett Hospital, The
Opportunity Available 53A

Matthew Thornton Health Plan
Opportunity Available 53A

Medical Center of Delaware
Opportunity Available 47A

Medical University of South Carolina
Opportunity Available 54A

Michael Reese Health Plan
Opportunity Available 54A

Merck Sharp & Dohme
Mefoxin 28A,29A,30A

Mount Sinai Medical Center
Opportunity Available 54A

Multigon Industries, Inc.
Doppler 8A

Murphy Medical Center
Opportunity Available 55A

National Institute of Health
Fellowship 26A

Orlando Regional Medical Center
Opportunity Available 55A

Ortho Pharmaceutical Corporation
All-Flex 6A
Ortho-Novum 7/7/7 .. Third Cover,Fourth Cover

Pennsylvania Hospital
Opportunity Available 55A

Permanente Medical Group, Inc.
Opportunity Available 55A

Roerig
Unasyn 31A,32A,33A,34A,35A,36A,37A,38A

Southboro Medical Group, Inc.
Opportunity Available 56A

St. Margaret's Hospital for Women
Opportunity Available 56A

Syntex Laboratories, Inc.
Femstat 39A,40A,41A,42A
Tri-Norinyl ..... Second Cover,1A,2A

University of California, Davis, Medical Center
Opportunity Available 56A

University of Mass. Medical Center
Opportunity Available 56A

University of Washington
Opportunity Available 57A

University of Wisconsin Hospital & Clinics
Opportunity Available 57A

Wright State University School of Medicine
Opportunity Available 57A

Upjohn Company, The
Provera 3A,4A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

21-and 28-day regimens

ORTHO-NOVUM 777 Tablets

norethindrone/ethinyl estradiol TRADEMARK

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

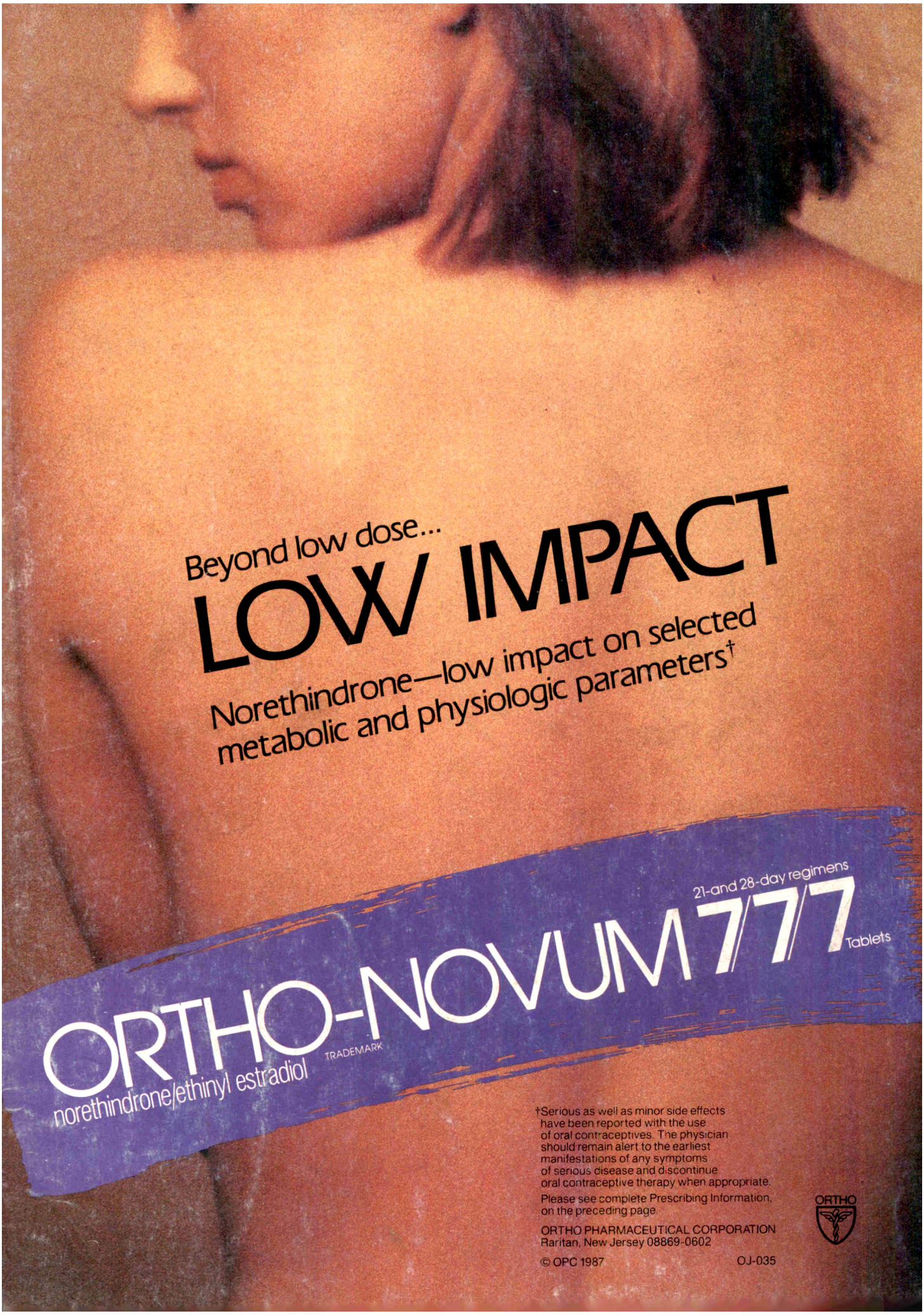
Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. Cases of hepatocellular carcinoma, although rare, have been reported in women taking oral contraceptives. Retrospective studies conducted in the United States and Britain report an increased risk of hepatocellular carcinoma in oral contraceptive users, particularly with increased duration of use. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **DRUG/LABORATORY TEST INTERACTIONS:** The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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INTERNATIONAL SYMPOSIUM ON CONTRACEPTION

Chairmen
Leon Speroff, M.D.
Egon Diczfalusy, M.D.

October 28, 1986
Singapore

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WYETH LABORATORIES, INC. and SCHERING AG, WEST GERMANY



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Contents

October
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PART 1: ORAL CONTRACEPTION

Introduction 1019
Leon Speroff, M.D.
Cleveland, Ohio

Contraception: An overview 1020
Jürgen Hammerstein, M.D.
Berlin, West Germany

Hormonal contraception: Benefits versus risks 1023
Joseph W. Goldzieher, M.D.
Houston, Texas

Metabolic effects of oral contraceptives 1029
Ulysse J. Gaspard, M.D., Ph.D.
Liège, Belgium

(Contents continued on page 4A)

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Contents continued from page 3A

Coagulation effects of oral contraception 1042
John Bonnar, M.D.
Dublin, Ireland

Progestogen potency in oral contraceptive pills 1048
Michael D. G. Gillmer, M.D.
Headington, Oxford, United Kingdom

Phasic approach to oral contraceptives 1052
Ralph W. Hale, M.D.
Honolulu, Hawaii

New progestogens in oral contraceptives 1059
Benno Runnebaum, M.D., and Thomas Rabe, M.D.
Heidelberg, West Germany

PART 2: ALTERNATIVE METHODS OF CONTRACEPTION

Introduction 1064
Egon Diczfalusy, M.D.
Stockholm, Sweden

Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs 1065
W. Elger, M.D., M. Fähnrich, S. Beier, Ph.D., Shi Shao Qing, M.D., and K. Chwalisz, M.D.
Berlin, West Germany, and Beijing, China

Recent developments in immunocontraception 1075
G. P. Talwar, D.Sc., and Amitabh Gaur, Ph.D.
New Delhi, India

Experiences with gossypol as a male pill 1079
G. Z. Liu, M.D., K. C. Lyle, Ph.D., and J. Cao, M.D.
Beijing, China, and New York, New York

Natural family planning 1082
J. B. Brown, D.Sc., L. F. Blackwell, Ph.D., J. J. Billings, M.D., B. Conway, B. Med. Sc., R. I. Cox, D.Sc., G. Garrett, J. Holmes, Dip. App. Biol., and M. A. Smith, Ph.D.
Victoria, Melbourne, and Prospect, New South Wales, Australia

A new delivery system for contraceptive steroids 1090
Sheldon Segal, Ph.D.
New York, New York

American Journal of Obstetrics and Gynecology

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volume 157 *number* 4 *part* 2 OCTOBER 1987

PART 1: ORAL CONTRACEPTION

Introduction

Leon Speroff, M.D.
Cleveland, Ohio

A decade's worth of evidence has helped quiet the storm of controversy that once surrounded the use of oral contraceptives (OCs). Our message to each other as clinicians and to our patients can now be a very positive one: The low-dose OC formulations available today not only offer nearly perfect efficacy but are also safer than ever before.

This message is bolstered by a series of reports presented in the first half of a Contraception Plenary Session at the XII World Congress of Fertility and Sterility in Singapore, October 28, 1986, the proceedings of which appear in this publication. The international experts who participated in this symposium accurately assessed the epidemiologic claims of an association between contraceptive steroid use and deep vein thrombosis, myocardial infarction, and stroke, and went on to indicate that there is no case for an OC–breast cancer link. The optimism of their epidemiologic reassessment is mirrored by current clinical evidence that the new low-dosage formulations represent a major step forward in enhancing the safety of hormonal contraception. The data presented at this symposium indicate that low-dose triphasic OCs exert no significant impact on metabolic and hematologic indices thought to predict cardiovascular risk.

We hope that the proceedings of this symposium will provide clinicians with reassuring new perspectives on the state of the art in oral contraception as well as with practical guidelines for patient care.

From Case Western Reserve University.

Contraception: An overview

Jürgen Hammerstein, M.D.
Berlin, West Germany

In this century, paramount progress has been made in many areas of medicine, not least in the field of contraception, with landmarks such as the introduction of intrauterine contraceptive devices (IUDs), hormonal contraceptives (OCs), and laparoscopic sterilization. The general optimism of the 1960s about overcoming the problems of global overpopulation with these new tools has subsequently been replaced by growing concern about the risks of contraception in general and of OCs especially. It was not before the 1980s that the benefit-risk balance of hormonal contraception turned out to be much more favorable than was formerly suspected. The wide variation from country to country in the prevalence of contraceptive methods has economic, political, religious, sociological, educational, and legal rather than medical reasons. Former misjudgments have led to a ban on valuable tools such as the sequential regimen or the use of 17-acetoxyprogestogen analogues as components in OCs, or to a ban on the licensing of depot progestogens in some countries but not in others. The recent decision of U.S. firms to cease production and sale of IUDs for legal reasons is unique and hopefully will not be imitated elsewhere. Just after the introduction of oral contraception, tremendous inventive dynamics led to the discovery of several new contraceptive principles. Most of these have reached the stage of clinical application and are being used satisfactorily, at least in some parts of the world. Later contraceptive concepts are still in the stage of experimentation and no breakthrough appears to be directly ahead. If contraception means more than just meeting individual wants—if it is also to be an instrument for handling the increasing problems of overpopulation—then much more must be done to deepen our knowledge of reproductive physiology, to popularize efficient means of contraception, and to make them available to everyone. Otherwise, all efforts to improve health standards throughout the world according to the "WHO Global Strategy for Health for All by the Year 2000" will be in vain. (Am J Obstet Gynecol 1987;157:1020-3.)

Key words: Contraception, intrauterine contraceptive devices, hormonal contraception, laparoscopic sterilization, benefit-risk balance, global overpopulation

In this century, we have been witness to paramount progress in many areas of medicine and not least in the field of contraception, with landmarks such as the introduction of the first practical intrauterine contraceptive device (IUD), the Gräfenberg ring, in the 1920s; the elaboration of the first oral contraceptive (OC) by Pincus, Garcia, and Rock in the 1950s; and the development of laparoscopic tubal sterilization in the 1960s.

As a result of these major advances, the belief became widespread in the scientific community that it should be possible to overcome the problems of overpopulation on our globe by means of these new tools. I well remember the great optimism of various experts around 1970 that new developments in fertility control would replace oral and intrauterine contraception within a decade or so. Unfortunately, this prophecy was not fulfilled, as everyone knows. Progress has been slow, no innovative breakthrough has taken place, and there have been several setbacks. What then is the situation of fertility control today?

From the Division of Gynecologic Endocrinology, Sterility, and Family Planning, Department of Obstetrics and Gynecology, Klinikum Steglitz, Free University of Berlin.

Patterns of use

From recent literature three major conclusions concerning the prevalence of modern methods of family planning worldwide may be drawn (Table I).[1, 2] First, sterilization has become most popular, which is true for either sex; second, the use of OCs has not increased during the last decade; and third, apart from China where more than two thirds of all IUDs are worn, the prevalence of intrauterine contraception appears to be decreasing rather than increasing in most countries.

The disparity in use prevalence of family planning methods between regions of the world, countries, and even cultural subgroups within countries is striking and has no counterpart elsewhere in medicine. For example, oral contraception is the preferred method of contraception in western Europe, as well as in various other countries. Intrauterine contraception is most popular in China, as already mentioned. Sterilization is the predominant means in the United States and India, whereas the traditional barrier and "natural" methods, backed up by induced abortion, have the highest prevalence in such different countries as the USSR and Japan.

Also the percentage of women who just do not use any kind of contraception despite urgent need differs

Table I. Estimated number of millions of couples worldwide using methods of contraception

	1970*	1977*	1984		
			Worldwide	*Developing countries*	*Developed countries*
Sterilization	20	80	137	115	22
OCs	30	55	55	26	29
Coitus with condom	25	35	37	11	26
IUD	12	15	70	58	12
Other	60	65	26	7	19
Total	147	250	325	217	108
No. of couples of reproductive age			797	599	198

*Not including China.

Table II. Oral contraception: Reduction of risks by modification

Method	*Inauguration*		*Introduction*	
	Year	*Country*	*Year*	*Country*
Low-dose pill, 50 μg estrogen (micropill)	1972	U.S.A. FRG	1973	Several
Triphasic low-dose regimen	1977	FRG	1979	FRG
Change of estrogen/progestogen ratio	Since 1978	Several	1980	Several
"Natural" instead of synthetic estrogens	1977	Sweden	—	
New progestogens	More than 2 decades ago	Holland U.S.A. FRG	1981-87	Several

FRG = Federal Republic of Germany.

widely from country to country. Thus in India 68% of women at risk are not covered by modern measures of contraception.[1] This lack of contraceptive responsibility is, however, true not only for people living in the developing world but also, to a minor degree, for those in developed countries, with an estimated 30% of women not taking advantage of efficient contraceptive methods. This percentage may be much higher in special subgroups. For example, 50% of the more than 1 million teenagers in the United States who become pregnant involuntarily every year have never used contraception before their first conception.

That acceptability of contraceptive measures may develop in divergent directions can be derived from a comparison between the use rate of OCs in the United States and the Federal Republic of Germany over the years. Concurrent with the first campaign against oral contraception in 1970, there was a slight setback in the use of OCs in the United States. The most drastic drop in OC use rate occurred in the mid-1970s, when the first reports of a causal association between OC use and myocardial infarction became known. Thereafter, the popularity of hormonal contraception in the United States never recovered, with the use rate having remained below 10% for the last several years. Similar campaigns in the Federal Republic of Germany, initiated by the media for scientific or sometimes ideological reasons, failed to have any major influence on the OC use rate, which has been stable at around 30% for 14 years.

Divergent developments have also taken place in other countries. For example, there was a time when no fewer than 45% of all Dutch women between 15 and 44 years of age were using hormonal contraceptives. This is equivalent to about two thirds of all women in the reproductive age group who actually need and/or use fertility control. In Japan, at the opposite extreme, OCs are not yet officially permitted. In this country the use rate of ovulation inhibitors never exceeded 3%.

Contraceptive prevalence is also changing with age. The situation in El Salvador and Thailand may be considered exemplary in this respect.[2] Oral contraception is of utmost importance for younger women but is replaced later in reproductive life by other methods, namely, by female sterilization.

Nonmedical influences

It can be taken for certain that nowadays scientific information on the state of the art in contraception, as well as practical instruction on the various methods used in family planning, are available to anybody who asks. Therefore, the wide variation from country to country in the prevalence of contraceptive methods

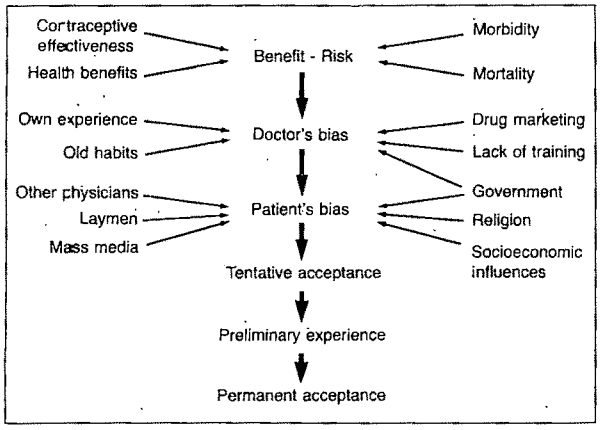


Fig. 1. The decision-making process: for or against the pill.

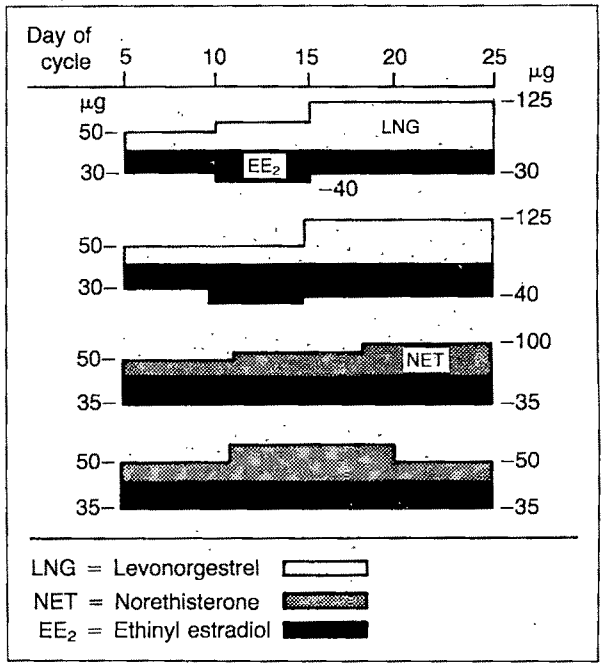


Fig. 2. Triphasic formulations presently available in the Federal Republic of Germany: Theme and variations.

must have economical, political, religious, sociological, cultural, and/or legal rather than medical reasons. Some of the grounds why proper medical counseling in this field is often biased are shown in Fig. 1. Socioeconomic and governmental influences are of highest importance in this respect, but drug marketing also plays some role.

The government impact arouses special concern, since decisions made by drug regulatory authorities are not always wise, and misjudgments will prevent persons from taking advantage of the progress of contraception, as has happened several times in the past. One such example is the withdrawal of all progestogens with a pregnane structure from oral contraception because of the wrongly interpreted breast tumor incidence in the beagle dog in the course of long-term toxicologic experiments. Revision of this more-than-15-year-old decision is mandatory, all the more since the 17-acetoxyprogestogens, in contrast to the estranes and gonanes, are devoid of any influence on lipoproteins.

Experience with the removal of the sequential formulations from the drug market in some countries has been quite similar: An increase in endometrial carcinomas had been observed only with one special preparation that contained the very weak progestogen dimethisterone for 5 days only. This regimen was thus equivalent to unopposed estrogen treatment, which notably can cause this type of tumor when given over long periods of time. The same has not been observed with any of the other sequential formulations. Nevertheless, they all had to be withdrawn from the market by decree in some countries.

To mention still one other example of misjudgment, which remains the secret of the Food and Drug Administration, is why depot progestogens are not permitted to be used as contraceptives in the country where they were developed. Although there are stringent medical indications to use them, in many places their benefits are withheld from the population without scientific justification.

Late in 1985, U.S. firms stopped the production and sale of IUDs for legal reasons. This is a completely new way of bringing progress to nought, leaving people with still fewer contraceptive alternatives than before. Hopefully, this step will not be imitated elsewhere.

New developments

In view of such retrograde progress, it is good to know of promising advances in the field, as was the case just after the advent of oral contraception and the rediscovery of the IUD. At that time, tremendous inventive dynamics led to the development of quite a few new contraceptive principles. Most of them, such as the minipill, depot progestogens, postcoital interception, subdermal implants, and vaginal rings (the latter two filled with progestogens), have since reached the state of clinical application and are being used satisfactorily at least in some parts of the world.

Whereas all of these new contraceptive principles were inaugurated before 1971, none of the innovative projects that were started after this date appears far enough advanced to come into general use soon, as discussed in the second part of this symposium.

Several minor changes in the mode of sex hormone application have been proposed. Although none of them is as important as the true innovations already mentioned, some have become quite popular, such as

the sequential and the step-up regimens. Others, like the once-a-month pill, the once-a-month injection, and the once-a-week pill, are used only in a few countries. Several additional modifications of oral contraception are already almost forgotten, as, for example, the Chinese paper pill, the French midcycle pill, and the British tri-cycle pill regimen.

The least spectacular but nonetheless most important line of recent development in oral contraception has been the worldwide endeavor to reduce the OC risk potential (Table II). This has led to a remarkable decrease in the hormone content of the pill, to the introduction of new contraceptive steroids, and to changes in the estrogen/progestogen ratio. In the latter respect, the triphasic regimens, of which several types are available (Fig. 2), have gained increasing attention in recent years. By these various means, OC-induced metabolic disturbances, which are often enough the starting point for side effects and serious complications, have been definitively reduced.

If contraception means more than just meeting individual needs, if it is also to be an instrument for handling the increasing problems of overpopulation, then much more must be done here and now to deepen our knowledge of reproductive physiology, to popularize the efficient means of contraception, and to make these methods available to everyone. There is no time to lose—it is less than 5 minutes til midnight. If the young generation, which constitutes nearly 50% of the population in some developing countries, behaves as their parents did after starting sexual life and reproductive activity, the problem of overpopulation will become disastrous. All developmental progress would be negated, with malnutrition, pauperization, and epidemic diseases being inevitable consequences. Then the optimistic "WHO Global Strategy for Health for All by the Year 2000" would not make sense any longer. We all should try to prevent this development, even if this means swimming against a torrential stream.

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Hormonal contraception: Benefits versus risks

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Epidemiologic studies, chiefly in English and U.S. populations, have generated concern regarding cardiovascular hazards associated with the use of oral contraceptives (OCs). A detailed analysis of these data suggests, however, that such studies have serious methodologic flaws. For example, the clinical diagnosis of deep vein thrombosis may be incorrect up to 50% of the time, rendering statistical analysis meaningless. The known cardiovascular risks of cigarette smoking may be amplified by OC use, but nonsmokers probably do not have an increased risk. Further, a number of studies in developing-world countries do not yield evidence of increased cardiovascular risks of OC use. Abnormal levels of serum lipids appear to affect cardiovascular (that is, myocardial infarction) risks in Western populations. Whether normalization of these levels improves the outlook is not entirely certain. The hormonal components of OCs are known to affect serum lipids, raising concern about possible long-term consequences. Newer low-dose OC formulations (such as triphasics) do not cause changes in serum lipids and therefore eliminate this putative risk. (Am J Obstet Gynecol 1987;157:1023-8.)

Key words: Hormonal contraceptives, benefits, risks

The effects of steroid hormones are ubiquitous, influencing as they do a great variety of endocrine and nonendocrine systems. Nowhere is this of greater clinical and epidemiologic importance than in the case of the estrogenic and progestational hormones used in contraceptive formulations, which were being used by upward of 50 million women around the world at the beginning of the present decade. In keeping with the magnitude of this usage, there is no class of drugs that has generated a larger literature on the possibilities of adverse reactions. In the space allotted to this article,

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Table I. Accuracy of clinical diagnosis of deep vein thrombosis, as checked on x-ray film or by use of radiolabeled fibrinogen, Doppler ultrasound, or impedance plethysmography

Author	*Year*	*Type of occurrence*	*No. with positive clinical diagnosis*	*Accuracy of clinical diagnosis*	
				False positive (%)	*False negative (%)*
Palko et al.[6]	1964	Postoperative	8	75	
Haeger et al.[7]	1967	Postoperative	512	46	
Flanc et al.[8]	1968	Postoperative	96	50	50
Haeger[9]	1969	Suspected DVT	72	54	
Kakkar et al.[10]	1969	Postoperative	132		50
Lambie et al.[11]	1970	Postoperative	111	25	66
Nicolaides et al.[12]	1971	Suspected DVT	97	47	
Yao et al.[13]	1972	Swollen legs	50	34	
Hicks[14]	1972	Postoperative	261		78
Tibbutt et al.[15]	1973	Suspected DVT	35	70	
		(1) Nurses, OC users	7	100	
		(2) All OC users	23	52	
Cranley et al.[16]	1976	Suspected DVT	124	46	
Hull et al.[17]	1976	Suspected DVT	269		22
Barnes et al.[18]	1978	Suspected VT			
		(1) OC users	54	83.3	$p = 0.05$
		(2) Nonusers	75	69.3	
Clarke-Pearson and Creasman[19]	1981	Suspected DVT	91	74	
Kierkegaard[20]	1982	Suspected DVT, OC users	25	64	

DVT = Deep vein thrombosis; VT = vein thrombosis.

it will not be possible to discuss the well-known hormonal effects of estrogens and progestational compounds, nor can one address the question of contraceptive efficacy, its origin, and the influence of external factors on its maintenance. Additionally, the establishment of an association between a drug and a rare adverse reaction (let alone proof of a causal relationship) is an issue in itself, challenging as it does the applicability of the commonly used techniques of epidemiology. This problem has been addressed with increasing concern by many investigators including my associates and myself[1-3]; although they cannot even be summarized here, it will be advantageous to bear in mind the uncertainties inherent in epidemiologic investigations in contrast to studies that are assessed by the known and tested criteria of experimental research.

The question of adverse effects is viewed quite differently in the case of an agent used to treat disease than in the case of one used to control fertility, even though the impact of unwanted, uncontrolled fertility may ultimately have a greater effect on our future than did all the epidemics of the Dark Ages. Moreover, the cultural and religious implications of fertility control add a dimension, not amenable to scientific logic, that places such concerns in an entirely different context.

In a status report, entitled "Oral contraceptives in the 1980s,"[4] no fewer than 713 references were cited, even though injectable and implantable delivery systems for contraceptive steroids were excluded from the review. It is evident, then, that important effects of contraceptive steroids on vitamins, other nutritional components, and energy systems (carbohydrate, protein, fat metabolism), on immunologic and related phenomena (for example, the decrease in first attacks of rheumatoid arthritis), and on the central nervous system, urinary tract, and cutaneous structures cannot be addressed at this time. Because of their importance in the medical as well as in the public mind, effects on the cardiovascular system and on oncogenesis will be the focus of attention.

Cardiovascular effects

Blood coagulation. There is an enormous literature on changes in blood coagulation factors observed in users of oral contraceptives (OCs) and on the existence and possible consequences of a "hypercoagulable state." Mammen[5] has admirably summarized this literature as follows: "Many conflicting test results have been reported, probably reflecting in part the technical difficulties . . . in assay systems. None of the data obtained seems to be specific for OC users . . . and none was found uniformly in all takers . . .. At this time, it is not known whether oral contraceptives do or do not trigger the hemostasis system to develop a thrombosis or by which mechanism they could trigger the event."

This is the first of two problems that becloud the question of the relationship of thromboembolic disease to the use of contraceptive steroids. The second is the reliability of the epidemiologic data that report a statistically significant association between deep vein

Table II. Deaths from cardiovascular disease by age, smoking status, and oral contraceptive use*

Smoking status	*Deaths by age group*					*Total*
	15-29 yr	*30-34 yr*	*35-39 yr*	*40-44 yr*	*>44 yr*	
Nonsmokers						
Controls	0	1	0	2	1	4
Current users	0	0	2	0	1	3
Former users	0	2	2	0	2	9
Smokers						
Controls	0	1	1	2	2	6
Current users	1	2	4	8	5	20
Former users	0	4	1	5	12	22
Total	1	10	10	17	23	64

*Experience in total woman years: controls, 138,700; current users, 99,000; former users, 84,800. Number of smokers and nonsmokers about equal. Data from Royal College of General Practitioners study, provided by C. Kay, M.D.

thrombosis and contraceptive steroid use. The purely technical problems complicating such epidemiologic research have been discussed at length elsewhere,[1-3] and this controversial issue is not likely to be settled in the near future. However, another methodologic issue of overriding importance has become apparent in the last few years. The application of sophisticated methods, such as intravenous radiolabeled fibrinogen, Doppler ultrasound, and impedance plethysmography, has shown that the clinical diagnosis of deep vein thrombosis (on which all epidemiologic studies have relied) is *grossly inaccurate*. Rates of failure to make the diagnosis clinically (compared with instrumental diagnosis) range from 22% to 78%, and the rate of false positive clinical diagnoses ranges from 25% to 83% (Table I). In one interesting study,[21] a positive clinical diagnosis was incorrect in 69% of cases—except when the patients were known to be OC users, in which case the clinical diagnosis was incorrect in 83%. Obviously, epidemiologic arithmetic based on such faulty information is useless, and as a result, we are completely unenlightened as to whether there is an association, causal or otherwise, between deep vein thrombosis and contraceptive steroid usage.

Myocardial infarction and stroke. On the other hand, retrospective case/control studies of myocardial infarction and stroke indicated a significant "relative risk" associated with use of OCs that was not likely to arise from diagnostic error. Prospective studies appeared to confirm the hazard. Then a confounding factor—smoking—was discovered. When the data were adjusted for this known cardiovascular hazard, the risk was found to be almost exclusively in heavy smokers. The difference in relative risk between nonsmoking pill users and nonusers of the same age group was *no greater than could easily occur by chance.*

Recent mortality statistics from the Royal College of General Practitioners, London, bear out this finding (Table II). The data base of this study is now very large: 138,700 women years in controls, 99,000 in current pill users, and 84,800 in former pill users. The small number of deaths (64) in this large population is noteworthy. The number of deaths in the various age groups is so small that most biometricians would be very cautious in drawing inferences about increased risk. Nevertheless, the association with smoking is inescapable.

Studies[22,23] carried out on this side of the Atlantic are also reassuring. The Walnut Creek Study,[23] sponsored by the National Institutes of Health, included 16,638 women (28% of them pill users) followed up for a total of 107,000 woman years. The investigators concluded, ". . . in a population of young adult healthy white middle class women the risks of OC use appear to be negligible."

Even more recently, Porter et al.[22] summarized data on 36,428 women years of pill user experience. They reported seven cases of stroke, six of them in nonusers, and no myocardial infarcts. They also found no relationship between estrogen dose and the incidence of clinically diagnosed thromboembolic disease.

When the supposed increased risks for myocardial infarction and stroke were first publicized, there was immediate and appropriate pressure to decrease the OC dose to the minimum that maintained contraceptive effectiveness, on the assumption that the hazards were dose related. A succession of formulation changes occurred, in the course of which the dose of the estrogen component, either ethinyl estradiol or mestranol, dropped from about 50 μg to the minimum effective dose, about 30 μg. (The chance of contraceptive failure with the low-dose formulations inevitably increases in the event of irregular drug intake or decreased drug bioavailability. Fortunately, the problem has turned out to be less serious than expected, and the effectiveness statistics on these formulations are astonishingly good.[24]) Some revisionists now claim that the current decreased estimate of cardiovascular hazards associated with oral contraception is due to the downward shift

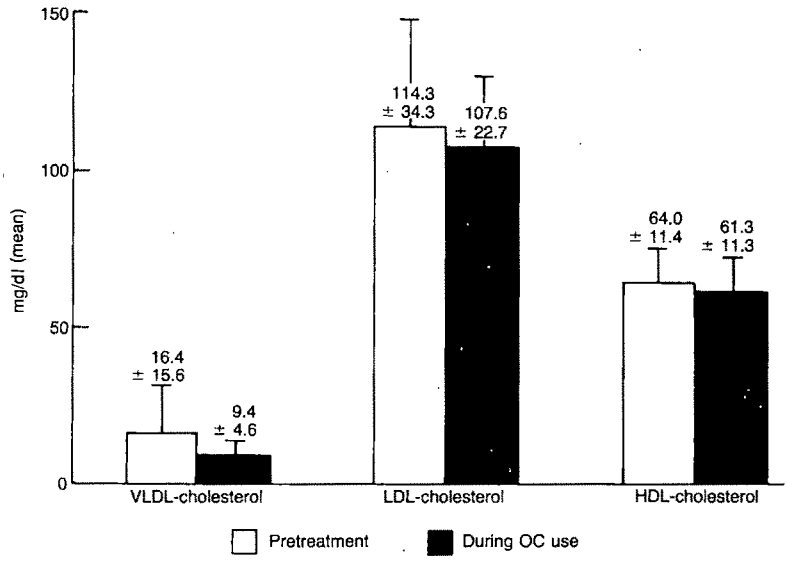


Fig. 1. Lipoprotein fractions with a levonorgestrel-containing triphasic OC. (Reproduced with permission from März W, Romberg G, Gahn G, Gross W, Kuhl H, Taubert HD. Effect of oral contraceptives upon serum lipoprotein pattern in healthy women. In: Rolland R, ed. Advances in fertility control and the treatment of sterility. Lancaster, England: MTP Press, 1984:99-106.)

in estrogen dosage. However, a number of studies show that this is not the case. In Denmark, for example, all pill users were abruptly switched to a low-dose pill in 1974. Stroke and myocardial infarction mortality statistics did not change, indicating either that lowering the dose does not decrease risk or that there was no measurable risk in the first place.

Blood lipids. Currently, there is appropriate concern about the possibility of ultimate cardiovascular effects from the influence of contraceptive steroids on blood lipids. Long-term studies on myocardial infarction seem to indicate that relatively minor changes in blood lipids can have significant effects on the incidence of myocardial infarction. For example, in one study a 19% reduction in coronary heart disease risk accompanied an 8% decrease in total serum cholesterol or an 11% decrease in low-density lipoprotein cholesterol.[25] Small increases in high-density lipoprotein cholesterol were also associated with a slight decrease in coronary heart disease risk. However, there is no unanimity among cardiovascular researchers as to the prognostic significance of various lipoprotein levels. (A recent British study even opined that high-density lipoprotein cholesterol levels were not predictive.[26]) Moreover, the expected advantages of altering lipoprotein levels in a "beneficial" direction are not convincingly demonstrable as yet; it would be beneficial for gynecologists to recognize the uncertain state of this research and not to take dogmatic statements by epidemiologists too seriously.

We must also recognize that the epidemiologic data on coronary risk are derived from a heavily male population; further, the women who sustained infarcts have been in the postmenopausal years. Kannel of the Framingham Study admitted several years ago that there had not been a single myocardial infarction in a premenopausal woman in that study. It is clear that such data cannot simply be extrapolated to the much younger and substantially different population of OC users; nevertheless, these data certainly justify current efforts to modify OC formulations so as to have as little effect as possible on plasma lipids (Fig. 1). While the current literature describing the effect of specific formulations on the lipoproteins and lipids is not entirely consistent, it is clear that reduction of dosage, change in proportion of the two steroids, and the choice of one progestational agent over another can modify or eliminate altogether the effect of hormonal contraceptives on plasma lipids and lipoproteins.

Neoplasms

Many types of neoplasia, especially cancers of reproductive or related organs, are affected by steroid hormones. Both endogenous and exogenous hormones can influence the observed incidence and prognosis of cancer. Thus, when steroid contraceptives were introduced, there were expressions of concern that these agents might either initiate or promote oncogenesis. Two decades of research have produced reassuring data, in spite of the long lag period inherent in carcinogenesis.

Endometrial cancer. Except for experience with one particular sequential OC (now withdrawn), the epidemiologic data are uniformly in agreement in estimating

a substantial decrease in endometrial cancer, the degree of benefit apparently increasing with duration of use. For those who have used steroid contraceptives for a period of 2 years or longer, there seems to be a (persistent) reduction in incidence of about 50%, resulting in an estimated 2000 fewer hospitalizations per year for this disease in the United States.[27]

Ovarian cancer. With respect to ovarian cancer, steroid contraceptive use appears to have the same type of protective effect as pregnancy. Ovarian cancer has a poor prognosis: Even with the best treatment, the 5-year survival rate is only 20%. In the United States, it accounts for 6% of all female cancer deaths. The decrease in ovarian cancer was estimated at about 50% in steroid contraceptive users, and the effect persisted for more than 15 years after first use. In the United States, birth control pill use averted about 1700 cases in women aged 20 to 54 in 1979 alone.

Cervical cancer. The effect of hormonal contraceptives on cervical cancer is unclear. There is much to suggest that cervical neoplasia is a virus-induced disease. It is clearly related to certain characteristics of sexual activity, particularly age at first coitus and number of partners. Epidemiologic studies that do not control for these factors[28] appear to be an exercise in futility.

Benign breast disease. Benign breast disease appears to be favorably influenced by steroidal contraceptive use: Despite more intensive medical surveillance, breast biopsies are performed half as often in contraceptive steroid users as in nonusers.[27] Women who use steroid contraceptives have a somewhat decreased risk for fibrocystic disease as a whole; the higher the progestational dosage, the lower the risk. This makes good endocrinologic sense. Patients whose biopsy specimens showed atypia, and controls had similar degrees of steroid contraceptive usage, while cysts or papillomatosis were half as common in users as in controls.[29]

Breast cancer. Numerous studies of steroid contraceptive use and breast cancer have failed to show a statistically significant association, except for one study[30] that was heavily criticized on methodologic grounds and was essentially discounted by a special Food and Drug Administration workshop on this subject. The recently published joint Centers for Disease Control/National Institutes of Health study—a monumental piece of work—also failed to uncover any risk.[31,32]

Melanoma. A 1977 report from California raised suspicions about an association of melanoma with steroid contraceptive use.[33] However, a number of studies in other localities have failed to confirm this association.

Benign liver tumors. An interesting situation arose with respect to benign liver tumors, which are extremely rare but present the risk of spontaneous rupture and hemorrhage. One careful case/control study showed a very strong increase in relative risk associated with increased duration of steroid contraceptive use.[34] Further studies were hampered by the rarity of these cases. More recently, a histopathologic study from the Armed Forces Institute of Pathology pointed out that one type of hepatic tumor (fibro lamellar) typically occurs in the age group likely to be using steroid contraceptives and that this age-related bias probably accounts for the statistical association.[35]

Pituitary tumors. Finally, we turn to the question of pituitary tumors and steroid contraceptive use—a problem that arose from the observation of so-called "postpill amenorrhea" and its association with pituitary microprolactinomas. A recent multicenter study of pituitary adenomas failed to establish a statistical association with contraceptive steroid use,[36] and another report[37] clarified the situation even further: In women with prolactinomas who had used these steroids for contraception, the relative risk was 1 (that is, a chance association), whereas in women who had used steroid contraceptives for regulation of menstrual problems the relative risk was 7.7 (95% confidence limit 3.7 to 17), clearly indicating the preexisting nature of the alleged association.

In the last analysis, the effects of contraceptive steroids on various organ systems are important (aside from intrinsic scientific interest) in the achievement of the desired therapeutic result as balanced against the hazards of their use. However, any benefit/risk assessment extends far beyond the limits of medical/scientific considerations and must include psychosocial, economic, and demographic considerations. The incredibly time- and effort-consuming studies that have been sponsored by the developed countries are highly relevant and justifiable in the context of their health systems; one should, however, question their relevance and applicability to the developing world, where maternal mortality is an overriding concern and postpartum thrombosis or myocardial infarction in women is almost unknown.

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Metabolic effects of oral contraceptives

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Combination oral contraceptives (OCs) are probably not an independent risk factor for cardiovascular disease but through their metabolic actions, may partly amplify the effects of known risk factors for cardiovascular disease. This review of the literature and our own data indicate that use of high-dose, progestogen-dominant OCs induces a potentially atherogenic lipoprotein profile (high low-density lipoprotein–cholesterol:high-density lipoprotein–cholesterol ratio), mostly attributable to the antiestrogenic action of the progestogen content of these OCs. In contrast, lower-dose combination OCs with reduced amounts of progestogens and slight estrogen dominance, either monophasic or multiphasic, produce strikingly fewer adverse effects on lipoproteins. Moreover, use of low-dose, as opposed to high-dose, OCs results in almost unchanged glucose tolerance, marginally increased or unchanged insulin and glucagon responses to glucose, and probably unchanged levels and activity of peripheral insulin receptors. Further in-depth studies of low-dose OC formulations are mandatory to ascertain reduced metabolic risk of these OCs. (Am J Obstet Gynecol 1987;157:1029-41.)

Key words: Combination oral contraceptives, risk-factors for cardiovascular disease, high-dose and low-dose progestogens, atherogenic lipoprotein profile, glucose tolerance

Numerous epidemiologic studies have indicated an increased frequency of cardiovascular disease, such as deep vein thrombosis and thromboembolism, ischemic heart disease and myocardial infarction, thrombotic and hemorrhagic strokes, and hypertension, in oral contraceptive (OC) users.[1] However, if other well-known risk factors for cardiovascular disease such as smoking, hypertension, increased age, diabetes, and hyperlipidemia are already present, the frequency of ischemic heart disease and stroke is markedly increased.[2,3] Accordingly, there is currently some debate as to whether the OC itself can be considered to be a primary risk factor or, rather, is an amplifier of the above-mentioned risk factors, making some women more vulnerable to cardiovascular disease.

Indeed, excess mortality in current and past OC users is essentially confined to women over the age of 35 who smoke. The Royal College of General Practitioners study reported significant excess mortality but other epidemiologic studies have not.[4] Further, Realini and Goldzieher[5] recently questioned and minimized a direct association between OC use and cardiovascular disease in a critical review of potential bias in these epidemiologic studies. Some observations suggest that, on the one hand, reducing the estrogen dose of combination OCs might appreciably decrease the incidence of venous thromboembolism[6] and that, on the other hand, reducing the progestogen content might decrease the incidence of nonvenous deaths (mainly from stroke and coronary heart disease).[7]

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Laboratory findings suggest that both the estrogen and progestogen components of OCs correlate, often in a dose-dependent manner, with alterations in blood coagulation and fibrinolysis, the renin-angiotensin-aldosterone system, prostaglandins (particularly the prostacycline-thromboxane balance),[8] glucose tolerance,[9] and lipid and lipoprotein metabolism,[1] and even changes in vessel wall structure and function.[10] These metabolic effects of OCs could potentially cause such clinical problems as thrombosis and atherosclerosis.

Reducing the untoward cardiovascular side effects of OCs is essential since these drugs are designed for widespread use by healthy women and with minimum medical supervision. To achieve this goal, exclusion of women with high-risk factors as candidates for oral contraception has been advocated, and new OCs with reduced doses of both estrogen and progestogen have been formulated. This article summarizes some recent data concerning the effects of new low-dose OCs on lipoprotein and carbohydrate metabolism.

Design of experimental studies of metabolic effects of OCs

Lowering the doses of estrogen and progestogen in OCs also decreases the amplitude of fluctuations of biologic parameters in the subjects studied. In that respect, it seems reasonable to resort to prospective and preferably comparative and randomized, either straight or crossover, studies of healthy volunteers under the age of 35 who have none of the usual contraindications to OC use and who are carefully matched for age, race, anthropometric measurements, smoking, diet, alcohol consumption, and exercise habits. Con-

comitant study of a matched control group not taking OCs but submitting to the same investigation protocol would be ideal.

Moreover, as far as lipoprotein studies are concerned, fasting samplings are mandatory. Samples should be collected on two or more standardized occasions during control pretreatment cycles, preferably in the second phase (luteal phase), then serially collected during 6 to 12 months of OC treatment and again during the second or third posttreatment spontaneous cycles. Burkman et al.[11] insist on a 6- to 12-month study duration because of slowly ongoing fluctuations of apolipoproteins during therapy. However, Deslypere et al.[12] have reported that the effects of long-term use of OCs on lipids do not differ from those predicted from short-term (6-month) studies.

Studies of carbohydrate metabolism, currently performed mainly with the use of highly standardized oral glucose tolerance tests, should have a duration of at least 12 months and preferably 2 or more years.[13] They should also take into consideration circannual changes in glucose tolerance,[14, 15] which may misleadingly obliterate drug-induced metabolic fluctuations. Although retrospective studies of metabolic effects of OCs such as those reported by Wahl et al.[16] or insufficiently standardized results such as those of Wynn and Niththyananthan,[17, 18] for instance, are interesting because they are related to rather large and different populations of OC users, they are hampered by numerous biases. Heterogeneity of the study groups; uncertainty and heterogeneity concerning the nature, dosage, or even succession of OCs used; and drastically diminishing numbers of subjects during the course of the study (perhaps reflecting dropouts due to side effects) can deeply alter the meaning of the results and the value of comparisons between OCs.

Finally, fluctuations in the biologic results obtained in all these studies should be carefully analyzed in relation to the methodologic procedures used.[19] Although random, non–drug-induced fluctuations of some parameters may be statistically significant, they may be devoid of clinical meaning. For example, spontaneous variations of circulating lipid concentrations are generally estimated to be ± 10%, and accordingly statistically significant variations should be associated with fluctuations of absolute values by >10%.

Lipoprotein metabolism

Lipoprotein metabolism and atherosclerosis. In the plasma, cholesterol (C) and triglycerides are carried by five lipoprotein fractions: chylomicrons, very-low-density lipoproteins (VLDL), remnants, low-density lipoproteins (LDL), and high-density lipoproteins (HDL).

Transport lipoproteins for triglycerides are chylomicrons and VLDL, mostly originating from the small intestinal mucosa and the hepatocytes, respectively. It is noteworthy that apolipoprotein B is a major structural component of both types of particles. These triglyceride-rich lipoproteins are peripherally submitted to hydrolysis in the presence of extrahepatic lipoprotein lipase. Subsequently, glycerol and free fatty acids are delivered to peripheral tissues while these lipoproteins become remnant particles, partly removed from the circulation by the liver and partly progressively converted to LDL particles, rich in apolipoprotein B and C. In fact, LDL accounts for about 70% of circulating C and finally supplies this lipid to peripheral cells that possess high-affinity receptors for apolipoprotein B. In contrast, HDL particles, synthesized according to various processes and probably mainly originating as discoid particles (nascent HDL) from the liver, are rich in apoproteins A_1 and A_2. In a complex process of maturing to HDL_3 particles (the heaviest fraction of HDL), these lipoproteins intervene in the uptake of free C from peripheral tissues and other sources, its esterification by lecithin-C acetyl transferase, and subsequent transfer into the particle core. This HDL particle becomes a spherical, transformed HDL_2 lipoprotein rich in phospholipid and C (about 20% of circulating C). It is now strongly believed that HDL particles play a major part in the reverse transport of C from peripheral tissues to the hepatocyte and its subsequent excretion into the bile. Hepatic hydrolysis of HDL_2 phospholipids by endothelial hepatic lipase may contribute to this process, activate catabolism of HDL_2, and potentially generate some HDL_3 particles. More details on lipoprotein metabolism can be obtained in recent reviews by Miller,[20] Nikkilä,[21] and Heller.[22]

Greater knowledge of lipoprotein metabolism and its defects has enabled us to better understand the epidemiologic studies that describe a strong association between hyperlipidemia and the development of atherosclerosis and its multiple complications such as coronary heart disease.[23, 24] In most Western communities with high average consumptions of saturated fat and C, the prevalence and severity of ischemic heart disease in men as well as in women correlates well with different risk factors such as age, smoking, obesity, hypertension, and, of course, hyperlipidemia. In fact, a low HDL-C level, probably related to suboptimal reverse C transport, is an excellent predictor of subsequent heart disease (negative correlation with atherosclerosis) and is a better predictor of ischemic heart disease than are increases in LDL-C. An increase in LDL-C is involved in excess deposition of C in peripheral tissues and arterial walls (positive correlation of LDL-C and, to a lesser extent, of total C with atherosclerosis). Moreover, decreased levels of the subfraction HDL_2-C correlate better with the frequency and se-

verity of ischemic heart disease than do HDL-C or HDL_3-C.[25] The association between hypertriglyceridemia (mainly VLDL-triglycerides) and ischemic heart disease, although well recognized, is not totally a direct one. The cardiovascular risk inherent to hypertriglyceridemia appears to be partly associated with the precursor-product relationships between VLDL and LDL and the large inverse correlation between VLDL and HDL, confirming that VLDL concentrations are elevated in situations in which HDL levels are low.[26] It is thus understandable that the concentrations of HDL-C (and HDL_2-C), LDL-C, VLDL-triglycerides and of the ratios HDL-C:total C, HDL-C:LDL-C, or apolipoprotein A_1:apolipoprotein B[27] are epidemiologically important considerations in assessing the risk of atherosclerosis.

Effects of sex steroids on lipoprotein metabolism. After puberty and before the menopause, women with normal genital activity have lower median levels of VLDL and LDL but more HDL and a higher HDL:LDL ratio than do men of the same age. This sex difference in HDL concentrations is totally attributable to the increased HDL_2 subfraction in women.[20, 21, 27] The male:female coronary disease sex ratio is accordingly >1.0. The sex differential in secretion of the sex hormones accounts for the sex differential in lipoproteins, which, in turn, accounts for decreased atherogenic risk and increased longevity in women.[27]

Exogenous estrogens administered orally in high doses can increase liver synthesis and release of VLDL, triglycerides, and apolipoprotein B. They also increase plasma HDL concentrations, mainly by increasing the HDL_2 subfraction, HDL–apolipoprotein A_1, and plasma apolipoprotein A_1.[28] This rise in HDL levels could be partly related to a suppressive effect of estrogens on endothelial hepatic lipase.[21] The effects of estrogens on LDL concentration are controversial, but a decrease in LDL-C levels in postmenopausal women has been described.[21]

Natural progesterone given orally in replacement dosages (200 to 300 mg/day) is almost devoid of any effect on lipoprotein metabolism.[29] However, synthetic progestogens used in OCs are 19-nortestosterone–derived and, as such, display not only progestogenic but also androgenic and antiestrogenic actions capable of greatly altering lipoprotein metabolism. These steroids decrease plasma VLDL and triglyceride concentrations possibly by inhibiting hepatic synthesis and release of these compounds.[30] They can therefore antagonize the estrogen-induced rise in VLDL and triglyceride concentrations. LDL-C levels are increased by 19-nortestosterone–derived progestogens. In contrast, HDL and its subfraction HDL_2 are highly sensitive to the inhibitory action of these progestogens with antiestrogenic androgenic activity, which also reduce plasma apolipoprotein A_1 and potentially increase apolipoprotein B concentrations.[31, 32] By comparison, it is interesting to note that the use of androgenic anabolic steroids such as stanozolol in postmenopausal women is associated with increases in LDL-C, dramatic decreases in HDL-C (−50% from baseline) and in the most antiatherogenic subfraction HDL_2 (−85% from baseline), and less prominent decreases in HDL_3.[33] This experimental reversion of female to male lipid profile may increase the atherogenic risk of the subjects so treated.[27]

Combination OCs contain not only potent synthetic estrogens (ethinyl estradiol or mestranol) but also 19-nortestosterone-derived progestogens, such as the estranes norethisterone, lynestrenol, ethynodiol, ethynodrel, or dimethisterone, and the gonanes (levo)norgestrel, desogestrel, and, in the near future, norgestimate or gestodene. For this reason, it is of paramount importance to investigate whether these combination estrogen-progestogen OCs can alter lipoprotein composition and concentration and thereby the atherogenic risk of OC users.

Effects of high-dose combination OCs on lipoprotein metabolism. Until rather recently, most of the literature on the suspected alterations in lipid metabolism associated with OC use was mainly concerned with products containing 1 to 10 mg of a progestogen and 50 to 150 μg of either ethinyl estradiol or mestranol. Increased total serum triglycerides and less prominent fluctuations in total C levels (mainly small increases) were reported in users of these high-dose OCs.[34] More recent analyses[16, 31] have shown that those OCs that are overly progestational (that is, they contain high amounts of norethisterone, ethynodiol diacetate, or norgestrel and standard amounts of ethinyl estradiol or mestranol) induce increases >50% in median concentrations of total triglycerides when compared with concentrations in control subjects not using OCs. In addition, they cause a significant 24% increase in median concentrations of LDL-C, also accounting for the observed elevation in total serum cholesterol levels. Wahl et al.[16] reported no significant changes in HDL-C with high-dose OCs containing norethisterone or ethynodiol but a significant 20% decrease with a combination OC containing a high dose of norgestrel (500 μg). This study combined in the same group women taking combination OCs containing only 1 mg of norethisterone and women taking the higher dose of 2.5 mg, which may partly explain the small variation in HDL-C concentrations when compared with those of nonusers. However, Bradley et al.[31] noted a 20% decrease in HDL-C levels in subjects using OCs containing 2.5 mg of norethisterone, a result quite comparable to that obtained with the OC containing high

Table I. Tentative summary of different studies of effects of diverse combinations of ethinyl estradiol and norethisterone on lipoprotein metabolism

Type of OC (μg/day) and investigators	*Total C*	*Triglycerides*	*LDL-C*	*HDL-C*	*HDL_3-C*
EE 50 + NE 250[16,31]	Increased	Increased	Increased	Decreased	—
EE 35 + NE 1000[11,35]	Increased	Increased	Increased or no change	Decreased or no change	—
EE 35 + NE 750 (triphasic)[40]	Increased	Increased	Increased	No change	—
EE 35 + NE 500[41,42]	No change	Increased or no change	No change	No change	—
EE 35 + NE 400[43]	Increased	No change	No change	Increased	Increased

EE = Ethinyl estradiol; NE = norethisterone. Unless indicated otherwise, all changes are statistically significant relative to baseline levels or versus control group, as stated by investigators.

*Borderline statistical significance.

doses of norgestrel. In women using an even more progestogen-dominant combination (ethinyl estradiol, 30 μg, plus levonorgestrel, 250 μg, equivalent to norgestrel, 500 μg),* Wynn and Niththyananthan[18] reported significant decreases in HDL_2-C (−20% as compared with control value) and in HDL_3-C (−10%) concentrations. Such changes in HDL_2 and HDL_3 subfractions were not observed with combinations of ethinyl estradiol or mestranol, plus norethisterone, 1 mg, but these preparations are hardly comparable with the levonorgestrel-containing OC since they are much less progestogen dominant.

Other combination or sequential high-dose OCs are overly estrogenic, containing high amounts of ethinyl estradiol or mestranol and lower doses or shorter administration periods of progestogens such as, for instance, norethynodrel or dimethisterone. These preparations significantly increase median triglyceride concentrations by 80% and HDL-C levels by 35%, when compared with control values, whereas they only marginally and nonsignificantly increase LDL-C levels. These observations are in good agreement with the experimental results described above and indicate that intake of high-dose progestogen-dominant OCs is associated with moderate hypertriglyceridemia and a potentially more worrisome increase in the LDL-C:HDL-C ratio. In contrast, estrogen-dominant OCs, although associated with marked hypertriglyceridemia, also induce a more "favorable" decrease in the LDL-C:HDL-C ratio, thereby decreasing, at least theoretically, the atherogenic risk. However, these overly estrogenic preparations are associated with excess morbidity and mortality mainly as a result of venous thromboembolism[6] and endometrial adenocarcinoma, and they have been withdrawn from the market.

As summarized by Miller,[20] the estrogenic effects on lipoproteins are opposite to those of progestogens so that the net response to combinations of these agents is determined by the doses of the particular steroids employed. Since the most convenient estimate of atherogenic risk might be the ratios of total-C:HDL-C or LDL-C:HDL-C, an increase in these ratios by exogenous hormones should be viewed with suspicion.

The data of Wahl et al.[16] and Wynn and Niththyananthan[18] aroused considerable controversy because they implied that all 19-nortestosterone–derived progestogens did not act identically on lipoprotein metabolism and alleged that (levo)norgestrel induced a more atherogenic lipid profile in long-term users.

In addressing this important point, the following arguments must be considered: (1) The retrospective studies of these groups[16, 18] had numerous biases, mentioned earlier, that did not allow comparison of the highly progestogen-dominant preparation containing norgestrel with the equally progestational combination OCs. Moreover, the data of Wahl et al.[16] are at variance with the study of Bradley et al.,[31] which reported a decrease in HDL-C levels with norethisterone high-dose combinations similar to that seen when the norgestrel high-dose combinations are used. This decrease in HDL-C is in agreement with other studies, including a recent multicentered study conducted by the World Health Organization with OCs containing lower amounts of both these progestogens.[35] (2) With regard to the potency and nature of norethisterone and (levo)norgestrel, both compounds are derived from 19-nortestosterone and bind directly with high affinity to progesterone and androgen receptors and to sex hormone–binding globulin. The effects of these steroids on pharmacokinetic parameters and receptor binding and their in vivo activity (estrogenic, androgenic, progestogenic) are largely similar.[36] However, although norethisterone, norethisterone acetate, and ethynodiol diacetate are roughly equivalent in potency, levonorgestrel is approximately five to 20 times as potent, depending on the type of effect considered.[37]

As stated by Fotherby,[1] the effect of a low dose of a high-potency progestogen should be roughly the same

*This combination is not available in the U.S.

HDL_2-C	HDL-C/total C	HDL-C/LDL-C	Apolipoprotein A_1/ Apolipoprotein B	Apolipoprotein A_1	Apolipoprotein B
—	Decreased	Decreased	—	—	—
—	No change	No change	Decreased	Increased	Increased
—	—	—	—	Increased	Increased
—	No change	No change	—	—	—
Increased*	No change	No change	—	—	—

as that of a high dose of a low-potency compound. This can be achieved with combination OCs containing (levo)norgestrel, since this progestogen powerfully inhibits gonadotropin secretion and hence gonadal function. The dose can therefore be drastically reduced in combination OCs without losing contraceptive effectiveness and the estrogen-progestogen balance of the preparation can be maintained because of its high antiestrogenic activity. In that way, the monthly dose of levonorgestrel in monophasic combinations has been reduced from 5.250 to 3.150 mg and even to 1.925 mg in a triphasic OC, which is a 63% decrease in progestogen content. In their study of the effects on lipoprotein metabolism of combination OCs containing degressive doses of levonorgestrel and accordingly characterized by moderately increasing ethinyl estradiol:levonorgestrel ratios, Larsson-Cohn et al.[38] concluded that ethinyl estradiol and levonorgestrel could be balanced so as to minimize undesirable effects on lipid metabolism. This also holds true for combination preparations with reduced doses of both ethinyl estradiol and norethisterone characterized by only minimal repercussions on lipoprotein metabolism (see below). (3) If there were a difference in "nature" between norethisterone and levonorgestrel, it would probably reside in a slight estrogenic activity of norethisterone already observable by its discrete keratinization effect on rat vaginal mucosa[36] and other estrogen-like effects.[39] Recent studies have shown that nonaromatizable metabolites of norethisterone exert typical estrogenic effects, such as induction of pituitary progestogen receptors.[39] No estrogenic metabolites of levonorgestrel have been identified so far, partly explaining why its antiestrogenic action is higher than that exhibited not only by norethisterone but also by desogestrel (see below). Its ability to depress plasma levels of sex hormone–binding globulin[36] and its ability to increase endothelial hepatic lipase activity[21] more so than other progestogens are able are two illustrations of its high antiestrogenic capability, which must be balanced by an adequate amount of ethinyl estradiol in combination OCs.

Effects of low-dose combination OCs on lipoprotein metabolism. Concern about increased cardiovascular risk in OC users has prompted the development of lower-dose combination OCs in the last decade. More studies of lipid metabolism, and particularly of lipid fractions that are potentially closely associated with atherogenic risk, have recently been conducted.

However, although many of these studies are prospective, they differ widely in protocol, duration of OC use, nature of the lipoproteins and lipid studied, statistical evaluation, and characteristics of the subjects tested. Consequently, all results must be interpreted with caution, keeping in mind that many of the changes recorded are small, although eventually statistically significant. They usually only reflect the summation of individual physiologic variations, especially if they are within the normal range. This caveat is particularly important in view of the small variations in lipid levels provoked by new low-dose OCs. A careful review of most of the recent studies devoted to that subject has been made by Fotherby.[1] Tables I (references 11, 16, 31, 35, 40-43) and II (references 16, 18, 31, 35, 38, 44-55) represent an attempt to synthesize the results obtained by different investigators and to indicate the statistically significant changes observed for relevant lipids and lipoprotein fractions and ratios, when there was a consensus among the studies. Otherwise, the discrepancy between results was noted.

Table I compares different preparations containing ethinyl estradiol and the estrane progestogen norethisterone, listed in order of decreasing steroid content. The roughly equal potency and metabolic effects of all progestogens of the estrane group (also including norethisterone acetate, ethynodiol diacetate, and lynestrenol) were taken into account.[37] Accordingly, attention was focused only on norethisterone-containing products. A comprehensive study by Powell et al.[56] of diverse preparations containing different doses of ethynodiol diacetate yields comparable results. In Table I, the high-dose combination OC containing ethinyl estradiol, 50 μg, plus norethisterone, 2500 μg, has been added for the sake of comparison with lower-dose preparations.

Table II compares preparations containing ethinyl estradiol and one of the gonane progestogens, levo-

Table II. Tentative summary of different studies concerning effects of diverse combinations of ethinyl estradiol and gonane progestogens on lipoprotein metabolism

Type of OC (μg/day) and investigators	*Total C*	*Triglycerides*	*LDL-C*	*HDL-C*	*HDL_3-C*
EE 50 + LNG 250[16,31,44]	Increased	Increased	Increased	Decreased	—
EE 30 + LNG 150[18,35,38,41-47]	No change	Increased or no change	No change	Decreased	No change
EE 32 + LNG 92 (triphasic)[38,45,48-52]	No change	Increased or no change*	No change	No change	Increased
EE 32 + GTD 79[49]	No change	Increased	No change	No change	—
EE 30 + DG 150[47,50-55]	No change	Increased or no change	No change	Increased or no change	Increased

EE = ethinyl estradiol; LNG = levonorgestrel; GTD = gestodene: DG = desogestrel. Unless indicated otherwise, all changes are statistically significant relative to baseline levels or versus control group, as stated by investigators.

*Borderline statistical significance.

norgestrel, desogestrel, or gestodene. Desogestrel, a 3-deoxo-11-methylene derivative of levonorgestrel, has roughly the same progestogenic and androgenic potency as levonorgestrel when used alone, but it has less antiestrogenic action when combined with the same dose of ethinyl estradiol.[57] The lesser antiestrogenic activity may be linked to intermediate hepatic metabolism of this compound from inactive desogestrel to active 3-ketodesogestrel. One of its intermediate metabolites, 3β-hydroxydesogestrel, binds to the estrogen receptor, as do other compounds with hydroxy groups at C-3 and C-17,[58] and might account for some of the estrogenic activity of this compound. With regard to gestodene, a Δ15-levonorgestrel derivative, its stronger inhibitory effect on ovulation allows it to be used in even smaller dosages than levonorgestrel can,[59] although its endocrine-pharmacologic profile is similar to that of levonorgestrel.[60] In Table II, the effects on lipid levels of the high-dose combination ethinyl estradiol 50 plus levonorgestrel 250 have also been indicated for the purpose of comparison with lower-dose products.

It must be stressed that data on ethinyl estradiol plus norethisterone combinations are still rather scarce, particularly for lower-dose formulations. Also, the duration of these studies is sometimes very short (for example, 7 weeks of treatment with ethinyl estradiol 35 plus norethisterone 400 in the study of Krauss et al.[13]). The consensus emerging from most studies is that reducing the estrogen and norethisterone content in OCs helps minimize increases in total C and triglyceride levels; even more interesting is the absence of any decrease in HDL-C concentration with low-dose compounds and the trend toward increased HDL-C levels with the lowest-dose combination, ethinyl estradiol plus norethisterone 400. This increase, which is probably related to a favorable ethinyl estradiol:norethisterone ratio, applies to the HDL_3-C and probably HDL_2-C subfractions as well.[43] At the same time, increases in LDL-C that were prominent with the OCs containing up to a mean daily dose of norethisterone 750 μg (in the triphasic ethinyl estradiol plus norethisterone formulation[40]) are no longer observable with the very low-dose preparations.[41, 43] Accordingly, the epidemiologically important ratios of HDL-C:total C and HDL-C:LDL-C do not change significantly with products containing <1000 μg of norethisterone per day, provided this progestogen is combined with at least 35 μg of ethinyl estradiol daily. Studies of apolipoproteins seem to indicate an increase in both apolipoprotein A_1 and apolipoprotein B with the low-dose combinations, but there are still too few data to allow any comment.

Much more information is available concerning the preparations containing gonane derivatives and ethinyl estradiol (Table II). Here again, the high-dose combination ethinyl estradiol 50 plus levonorgestrel 250 clearly induces changes in the lipoprotein profile that closely resemble those induced by ethinyl estradiol 50 plus norethisterone 2500. The well-known preparation ethinyl estradiol 30 plus levonorgestrel 150, which was probably the earliest available lower-dose combination (about 1974), has been extensively studied, and most references can be found in Fotherby's review.[1] Compared with the high-dose preparation, this OC does not increase total C and has no effect on or only slightly increases triglyceride levels. With ethinyl estradiol 30 plus norgestrel 300, the LDL-C decrease (−24% with the high-dose product) is no longer observed or is only borderline, and the 20% decrease in HDL-C levels seen with the high-dose OC is now only about −10% from baseline, probably because of a simultaneous moderate decrease in HDL_2-C.[43] Apolipoprotein B levels are still somewhat increased. Consequently the ratios HDL-C:total C, HDL-C:LDL-C, and apolipoprotein A_1:apolipoprotein B are only slightly decreased, indicating, at least theoretically, a reduction in the atherogenic risk when compared with higher-dose progestogen-dominant OCs. This trend toward lesser OC-induced changes in lipid profile is further exem-

HDL_2-C	*HDL-C/total C*	*HDL-C/LDL-C*	*Apolipoprotein A_1/ Apolipoprotein B*	*Apolipoprotein A_1*	*Apolipoprotein B*
—	Decreased	Decreased	—	—	—
Decreased	Decreased	Decreased or no change	Decreased	No change	Increased
No change	No change	No change	No change	Increased or no change	Increased or no change
—	No change	Increased*	No change	No change	Increased
No change	No change	Increased or no change	Increased	Increased	Decreased or no change

plified by studies of a triphasic combination containing mean daily doses of ethinyl estradiol 32 plus levonorgestrel 92. No changes in lipid and lipoprotein concentrations are observed,[52] except for a slight increase in HDL_3-C, although this rise is not reflected in a concomitant increase in HDL-C[48]; apolipoprotein A_1 and B levels are both slightly increased but not enough to provoke changes in the apolipoprotein A_1 : apolipoprotein B ratio.[38, 45, 48, 50-52, 61-63] A further decrease in the progestogen dose has been achieved in a new triphasic OC containing a mean daily dose of ethinyl estradiol 32 μg plus gestodene 79 μg, which is a slightly more estrogenic combination than the ethinyl estradiol plus levonorgestrel triphasic. In an ongoing 12-month prospective study of this new triphasic, Gaspard et al.[49] have observed only marginal changes in lipid and lipoprotein concentrations, with slight increases in total triglycerides and apolipoprotein B levels.

The monophasic combination of ethinyl estradiol 30 plus desogestrel 150 has also been widely studied, and there is a consensus (review in reference 1) that this formulation is more estrogen-dominant than is the combination containing ethinyl estradiol 30 plus levonorgestrel 150, the triphasic ethinyl estradiol plus levonorgestrel, and the triphasic ethinyl estradiol plus gestodene. It probably also is more estrogenic than all low-dose preparations containing ethinyl estradiol plus norethisterone. Consequently, about half of the studies concerning this OC report moderately increased total triglyceride levels; they also show unchanged or slightly increased HDL-C levels (about +5% to +10% from baseline), which are mainly accounted for by an increase in HDL_3-C[48] but probably also by a slight increase in HDL_2-C. Apolipoprotein A_1 levels increase and apolipoprotein B levels decrease slightly during intake of this OC.[52] The epidemiologically relevant lipoprotein and lipid ratios either do not change or increase slightly in, at least theoretically, an antiatherogenic direction. In our opinion, some concern should be raised, however, concerning the slightly excessive estrogen-dominant balance of this preparation. A consensus is emerging that for all the combination OCs reviewed, reducing the doses of both the 19-nortestosterone–derived progestogen component and the estrogen component leads to lesser changes in all measured parameters of the lipid profile. The concentrations of LDL-C are clearly proportionally higher in women treated with OCs that either contain the highest doses of progestogen or have the lowest estrogen : progestogen ratio. Triglyceride levels increase more readily with OCs containing higher amounts of estrogens or containing less antiestrogenic and/or very low-dose progestogens. HDL-C concentrations increase with higher doses of estrogens or with lower doses of progestogens and do not change with the more balanced preparations.[44] Review of the studies of low-dose OC use show that, although many lipid fraction changes are observed after 3 months of treatment, this is not a general rule, probably because changes in apoproteins proceed at a slower rate. More relevant information on lipid profiles during OC use comes from studies of 6 to 12 months' duration.[1, 11, 12] In contrast to previous assertions,[16, 18] reduction of the dose of *all* progestogens (even the more active ones, such as levonorgestrel or desogestrel, or the most antiestrogenic, such as levonorgestrel), provided that the estrogen : progestogen ratio is well adapted, helps minimize the repercussions on lipoprotein metabolism. This was well demonstrated by Larsson-Cohn et al.[38] in 1981 and again by further studies coming from different centers.[45, 52]

Carbohydrate metabolism

Carbohydrate metabolism and vascular disease. Since the first report of Waine et al.[64] in 1963 indicating a possible adverse effect of OC use on carbohydrate metabolism, many studies have investigated the extent of the potential alterations caused by different steroid types and dosages in both combination and progestogen-only OCs.

Diabetes mellitus is a well-documented independent risk factor for ischemic heart disease and cardiovascular death[65]; this risk is also partly mediated by the associ-

ation of diabetes with hyperlipidemia and hypertension and may be greater in diabetic women than in diabetic men. Moreover, impairment of glucose tolerance[66] and hyperinsulinism[67] may be associated with an increased risk of arterial disease. The plasma insulin response to a glucose load may be a sensitive indicator of the risk of arterial disease.[68] Since such abnormalities of glucose metabolism may be observed in OC users, particularly if additional risk factors such as obesity, family history of diabetes, age, and antecedents of gestational diabetes are present, it is essential to evaluate how risk may be minimized in OC users by proper drug and patient selection.[69]

Effects of sex steroids on carbohydrate metabolism. Some studies seemed to show an impairment of glucose tolerance during the luteal phase of the menstrual cycle—and hence partly in relation to progesterone secretion (review in reference 70). However, it is now well established, by means of sophisticated techniques, such as the euglycemic hyperinsulinemic glucose clamp, that glucose tolerance and insulin sensitivity are identical in both the follicular and the luteal phases of the cycle.[71]

Estrogens alone do not worsen diabetes and may even improve hyperglycemia in non–insulin-dependent diabetes.[70] Administration of synthetic estrogens, even in high doses up to 500 μg/day, does not consistently affect glucose tolerance or insulin response to a glucose load in healthy young women.[72] With synthetic estrogens contained in OCs, and particularly with higher doses (50 to 150 μg/day), a fall in the fasting plasma glucose level, a decrease in glucose tolerance mainly during the first hour after a glucose load, and a reduction of the early insulin response with subsequent enhanced insulin secretion have been repeatedly described.[73] These effects have been attributed to a direct action of estrogens on the liver and A and B cells of pancreatic islets, resulting in an overall decreased portal secretion of insulin and glucagon with an increased insulin:glucagon ratio. Impaired gluconeogenesis, reduced glycogenolysis, and increased liver glycogen storage may ensue,[74] explaining the fall in the fasting blood glucose level observed in OC users.

High doses of intramuscular progesterone (300 to 400 mg/day) equivalent to endogenous progesterone secretion during the last trimester of pregnancy, rapidly induce a decrease in glucose tolerance.[75] Tolbutamide tests performed concurrently in progesterone-treated subjects provoke an enhanced insulin response with subsequent blunted hypoglycemia.[73] These progesterone-induced changes in carbohydrate metabolism are typical of insulin resistance. Additionally, animal studies clearly show a direct effect of progesterone and progestogens on insulin release from pancreatic islets.[76]

Synthetic progestogens, administered alone even in small amounts and by the oral route, have the same potential as progesterone for impairing glucose tolerance in spite of hyperinsulinism. This has been well demonstrated in a series of studies by Spellacy et al.[77] in women receiving for 12 or more months low doses of either a 17-acetoxyprogesterone—derived progestogen (megestrol acetate, 0.5 mg/day) or one of the 19-nortestosterone–derived progestogens norethisterone (0.35 mg/day), ethynodiol diacetate (0.25 mg/day), or norgestrel (0.075 mg/day).[78-80] It is, however, obvious from these studies and from others that progestogens derived from 17-hydroxyprogesterone have a lower potential for impairing glucose tolerance and increasing insulin resistance than do the more potent 19-nortestosterone derivatives.

The mechanism of insulin resistance resulting from use of either combination or progestogen-only OCs is still poorly understood. Recent observations of Seed et al.[81] relate at least part of this resistance to potentially reduced levels of hepatic insulin receptors, resulting in decreased hepatic uptake of insulin; however, no direct evidence is currently available. A decrease in the number and affinity of peripheral insulin receptors, such as monocyte or erythrocyte receptors, has been postulated in subjects receiving progesterone, testosterone, or derived steroids in order to explain decreased insulin sensitivity.[82] However, convincing results in this field are still lacking, and our group has been repeatedly unable to observe any change in erythrocyte insulin receptor level and affinity in women receiving monophasic or sequential OCs containing ethinyl estradiol and desogestrel and triphasic OCs containing ethinyl estradiol and levonorgestrel or gestodene for 6 to 12 months.[15,49] Indeed, significant postreceptor changes in intracellular glucose metabolism may potentially contribute to insulin resistance in OC users.

Effects of high-dose OCs on carbohydrate metabolism. The numerous studies devoted to this subject have used different methods in very different populations, and the results frequently appear conflicting. From a review by Kalkhoff[70] in 1975, it appeared, however, that the different effects of combinations of estrogens and progestogens on glucose tolerance and insulin resistance are directly related not only to the respective amounts of these steroids but also to the type of progestogen used. Other important contributors to potential deterioration of glucose tolerance in OC users were preexisting abnormalities of glucose tolerance, such as impaired glucose tolerance or diabetes mellitus, age, obesity, and a familial history of diabetes or previous gestational diabetes. In a large prospective study, Phillips and Duffy[83] found that OC users had significantly lower fasting blood glucose levels and higher (by 11 mg/dl) 1-hour serum glucose levels than those of age-matched nonusers. However, past users, regardless

Table III. Changes in carbohydrate metabolism associated with ≥6 months' use of low-dose combination OCs

Type of OC (μg/day) and investigators	*Length of study (mo)*	*Blood glucose level*	*Plasma insulin level*
Estrane progestogens			
EE 35 + NE 1000[35]	12	Increased	ND
EE 35 + NE 750 (triphasic)[87]	12	No change	No change
EE 35 + 500[88]	12	No change	No change
EE 35 + NE 400[89]	12	No change	No change
Gonane progestogens			
EE 30 + LNG 150			
Study a[90]	12	No change	No change
Study b[17]	20	Increased	Increased
Study c[35]	12	Increased	ND
EE 32 + LNG 92 (triphasic)			
Study a[30]	6	Increased	Increased
Study b[15]	6	Increased*	Decreased
Study c[91]	12	No change	No change
Study d[61]	6	No change	No change
EE 32 + GTD 79 (triphasic)[91]	12	No change	No change
EE 30 + DG 150			
Study a[15]	6	No change	Decreased
Study b[62]	6	Increased	Increased

EE = Ethinyl estradiol; NE = norethisterone; LNG = levonorgestrel; GTD = gestodene; DG = desogestrel; ND = not determined.

*Borderline statistical significance.

of the duration of use, experienced the same glucose response as that of women who had never used OCs.

There is a consensus that glucose tolerance is more impaired with overly estrogenic high-dose OCs. There is also agreement that standard dose OCs (containing 50 μg of ethinyl estradiol per day) in combination with a pregnane progestogen, such as chlormadinone acetate or megestrol acetate, cause significantly less impairment of glucose tolerance than do combinations containing high doses of estrane or gonane progestogens.[70-73] Perlman et al.[84] reanalyzed glucose tolerance data from the large prospective Walnut Creek Contraceptive Drug Study by multiple linear regression analyses in order to define relationships between specific progestogen and estrogen types and dosages and parameters of glucose tolerance. As expected, the mean 1-hour and 2-hour glucose values after an oral glucose load were higher in OC users than in nonusers and impaired glucose tolerance was observed twice as frequently in users as in nonusers. Regression slopes for the three structurally similar estrane progestogens norethisterone, norethisterone acetate, and ethynodiol diacetate indicated a 5 to 10 mg/dl increase in serum glucose per milligram of progestogen exposure, for both 1-hour and 2-hour test results, and the slopes reflected an 18 to 35 mg/dl increase per milligram of norgestrel. This correlates very well with the higher activity of this progestogen, which was discussed earlier,[37] and indicates the need to use low doses of levonorgestrel in combination OCs containing this steroid.

However, as recently observed by Vexiau et al.[85] in eight women presenting with impaired glucose tolerance, 12 months of use of ethinyl estradiol 50 μg plus norgestrel 500 μg results not only in insulin resistance but also in unexpected and paradoxical correction of glucose intolerance probably as a result of the OC-induced hyperinsulinism. Nevertheless, use of high-dose OCs in women with previous gestational diabetes or with impaired glucose tolerance usually results in rapid deterioration of glucose tolerance in more than half of the subjects. This deterioration progressed to diabetes mellitus in 44% of the patients in Kalkhoff's review,[70] probably because of the inability of the pancreas to further secrete insulin in response to the diabetogenic stress induced by the pill. In contrast, large prospective studies of women using OCs, even in high doses, have not been able to delineate a significantly increased risk of developing diabetes mellitus when these women are compared with nonusers.[86] Moreover, in a large prospective follow-up study (over a mean of 8.5 years) of women with glucose intolerance, high-dose OC use did not induce permanent changes in glucose status, although increased glucose intolerance was observed in women who were older, were obese, or had a positive family history of diabetes.[13]

Effects of low-dose OCs on carbohydrate metabolism. Most of the prospective studies of low-dose combination OCs are summarized in Table III (references 15, 17, 35, 50, 61, 62, 87-91). Changes in blood glucose and insulin levels, although statistically significant, are usually within the normal range, and most of the fluctuations reported do not appear to be clinically signif-

icant. This is particularly the case for combination OCs containing low doses (less than 1 mg a day) of estrane progestogens, which seem to induce fewer changes in carbohydrate metabolism than do the corresponding progestogen-only minipills.[78-80] In the study of Rabe et al.,[87] the triphasic preparation containing mean daily doses of ethinyl estradiol 35 plus norethisterone 750 μg caused a transient increase in 1-hour and 2-hour glucose values and in plasma insulin levels when compared with basal pretreatment levels. At 12 months, however, the differences in glucose tolerance and insulin response were no longer statistically significant. No change in carbohydrate metabolism was apparently observed with low-dose combination OCs containing either 500 μg or 400 μg of norethisterone.[88,89]

The effects of carbohydrate metabolism of the combination preparation containing the intermediate dose of levonorgestrel 150 μg/day are controversial. Ahren et al.[90] did not observe any adverse effect with this OC, in contrast to the findings reported in the World Health Organization multicenter study[35] and in Wynn's study.[17] Wynn observed a deterioration of glucose tolerance that increased with time for up to 36 months of study. An accompanying increase in insulin response developed during OC use, but it could not prevent an increase in the ratio of incremental glucose area : incremental insulin area. Impaired glucose tolerance developed in six subjects. However, the major drawback of this study is an uncommonly high dropout rate: 210 women began the study, but only 17 remained under investigation after 3 years. Consequently, these results must be regarded as circumstantial and, in our opinion, no firm conclusion can be drawn from this study. In a preliminary study of a combination monophasic OC containing a similar dose of desogestrel (150 μg a day), Schijf et al.[62] reported statistically significant increases in blood glucose and plasma insulin levels after 6 months of treatment. However no pathologic values were recorded.

The same observation was also reported in a study of a triphasic OC containing a low mean daily dose of levonorgestrel.[50] However, these data are not confirmed by a careful 12-month prospective study,[91] in which we compared the treated subjects at 0, 6, and 12 months of use with a group of matched nonusers. No change in glucose tolerance was recorded in any individual during treatment, and a slight increase in insulin response was only transiently observed in the sixth month of study. Additionally, no changes in insulin erythrocyte receptor number or affinity were recorded. These results are at variance with a previous 6-month study,[15] in which we observed a slight decrease in glucose tolerance associated with a decrease in insulin response when compared with pretreatment levels. This difference could be attributed to circannual changes in glucose-insulin homeostasis, as previously observed by Reinberg et al.[14] The protocol of our subsequent study was accordingly modified to avoid this physiologic interference. Skouby et al.[61] in a recent study did not observe any effect of the levonorgestrel-containing triphasic on carbohydrate metabolism. In a new triphasic OC containing a minimal mean daily dose of 79 μg of gestodene, the balance is slightly more estrogen dominant than in the triphasic containing levonorgestrel. In women using this OC, a slight but significant decrease in glucose tolerance is observed at 6 months, with a subsequent full recovery at 12 months. On the other hand, no change in insulin response when compared with the baseline value was recorded throughout the 12 months of study (Gaspard UJ, Lepot MR, Jaminet C, Jandrain B, Lefebvre PJ. Carbohydrate metabolism in women receiving two low-dose triphasic oral contraceptives for one year. Unpublished data.)

Thus decreasing the gonane progestogen dose in OCs is associated with reduced effects on carbohydrate metabolism. With the triphasic formulations, changes in glucose tolerance are virtually nonexistent. With the gestodene-containing triphasic, induced insulin resistance, which seems to be the most sensitive effect of progestogens on carbohydrate metabolism, is completely absent. In addition, it is noteworthy that in women with previous gestational diabetes who received the levonorgestrel triphasic for 6 months, the glucose, insulin, and the glucagon responses to oral glucose remain unchanged during the whole treatment period.[61]

Comment

Analysis of epidemiologic data concerning the association of cardiovascular disease with OC use suggests that OC use is probably not an independent risk factor but that it can amplify the deleterious effects of other direct risk factors. Risk factors for atherosclerosis that may be directly influenced by OC intake are altered lipoprotein metabolism (eventually resulting in decreased HDL-C, HDL_2-C, and apolipoprotein A_1 concentrations and/or in increased LDL-C, total C, apolipoprotein B, and triglyceride levels) and altered carbohydrate metabolism (characterized by impaired glucose tolerance and hyperinsulinism caused by insulin resistance). In fact, adverse effects on these metabolic parameters have been repeatedly described with the use of high-dose, progestogen-dominant OCs. Their formulations accordingly have been carefully adapted in a search for OCs with the least possible effects on diverse metabolic end points. Data emerging from new and ongoing prospective studies show definitely less impact of new OC formulations on metabolism. Low-dose combination OCs containing a daily dose of ≤35 μg of ethinyl estradiol in association with a minimal amount of estrane or gonane 19-nortestosterone progestogen (equivalent to ≤750 μg of norethisterone and to ≤100 μg of levonorgestrel),

preferably with a slight estogen dominance, seem best devised for respecting metabolic neutrality. The assumption that levonorgestrel derivatives are "too potent" is refuted by the observation of favorable metabolic results obtained with OCs containing very low doses of these progestogens, which compare equally well with results obtained with ethinyl estradiol/estrane preparations.

However, because of the appreciable metabolic changes that can occur with only slight alterations of OC formulation, careful evaluation of the metabolic effects of each new OC preparation is mandatory. The best approach to assessing effects on lipoprotein metabolism involves not only recording changes in levels of lipoproteins or even their subfractions but also analyzing apoprotein and lipid content and composition for each lipoprotein. Indeed, OC-induced changes in lipoproteins,[91] which are not reflected in a simple lipid or lipoprotein level analysis, can greatly alter lipid metabolism. In addition, before these low-dose formulations truly can be considered neutral, it will be necessary to gather more data on alteration of fatty acid composition, on key enzymes of lipid metabolism (for example, lecithin-C acetyl transferase, hepatic lipase), and on lipid and lipoprotein synthesis and metabolic rate during OC use.

With respect to carbohydrate metabolism, a better appreciation of insulin resistance is necessary, as are continued studies of its mechanism of appearance and development during OC use. Determination of C-peptide levels, in addition to insulin concentrations, appreciation of insulin receptor levels, and the use of glucose clamp–related techniques may potentially help to quantify more accurately the metabolic burden imposed by OCs, even in low dosages. Finally, it must be stressed that beyond their effects on carbohydrate and lipid metabolism, OCs display many other actions. These actions may have even more important implications with respect to cardiovascular disease and certainly deserve in-depth continuing study.

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Coagulation effects of oral contraception

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In Europe and North America, estrogen/progestogen oral contraception has been associated with an increase in venous thromboembolism, myocardial infarction, and stroke. These hazards are found mainly in smokers and in women over the age of 35. Venous thromboembolism appears to correlate with the estrogen dosage, and the arterial complications with both the estrogen and progestogen components. Blood coagulation and vascular thrombosis are intimately related. Estrogen/progestogen oral contraception affects blood clotting by increasing plasma fibrinogen and the activity of coagulation factors, especially factors VII and X; antithrombin III, the inhibitor of coagulation, is usually decreased. Platelet activity is also enhanced with acceleration of aggregation. These changes create a state of hypercoagulability that, to a large extent, appears to be counterbalanced by increased fibrinolytic activity. Studies of the oral contraceptives in current use show that the coagulation effects depend on the dosage of estrogen and the type of progestogen used in combination. Current research is aimed at finding the estrogen/progestogen formulations that induce the least changes in the coagulation system and other physiologic processes. In this respect, the new low-dose formulations are a major step forward and should reduce the risk of vascular thrombotic complications. (Am J Obstet Gynecol 1987;157:1042-8.)

Key words: Estrogen/progestogen contraceptives, thromboembolism, myocardial infarction, stroke, dosage of estrogen, type of progestogen

After 25 years of extensive use of oral contraceptives (OCs), the most important concern about the effects of these substances on women's health is the increased risk of cardiovascular disease, specifically, venous thromboembolic disease, myocardial infarction, and stroke.[1,2] These hazards increase especially in older patients and with smoking. The information on the vascular effects of OCs is derived almost entirely from the epidemiologic research carried out in Europe and in the United States and is based on preparations that contain one of several progestogens in combination with 50 μg of mestranol or ethinyl estradiol. These findings therefore relate to countries in which the incidence of cardiovascular disease is high; thus they may not be relevant to countries in which the incidence of these diseases is low. In this context, it should also be noted that important differences in coagulation and hemostatic tests have been shown in healthy women from different ethnic groups and geographic locations,[3] and that epidemiologic studies from certain countries do not demonstrate an increased incidence of vascular disease in association with oral contraception.[4]

Since 1974, important information on the effects of OCs on morbidity and mortality has come from three cohort studies that involved more than 80,000 women, half of whom were using OCs (The Royal College of General Practitioners Oral Contraception Study, the Oxford/F.P.A. Contraceptive Study, and the Walnut Creek Contraceptive Drug Study[2]). The Royal College of General Practitioners data suggest that the morbidity from vascular complications was increased fourfold; most deaths were caused by ischemic heart disease or subarachnoid hemorrhage and were concentrated in women over the age of 35 or older and in smokers (Table I). In general, among women of reproductive age the risk of death from a vascular complication attributable to OCs was 22 to 24 deaths per 100,000 women per year in both current and past users.

The effect of oral contraception on the incidence of venous thromboembolic disease is shown in Table II. Oral contraceptive use was considered to be responsible for 19 cases of overt venous thromboembolic disease per 10,000 current users per year; just over half of these cases were superficial leg vein thrombosis. The relative risk of idiopathic deep vein thrombosis and pulmonary embolism was approximately fourfold, and the relative risk of postoperative venous thrombosis was twofold.

Pathogenesis of vascular complications

The pathogenesis of venous thromboembolism in OC users may relate to increases of blood coagulability (including changes in the levels of coagulation factors and inhibitors and altered fibrinolytic and platelet activity) and to changes in the structure of veins and arteries (such as endothelial proliferation and changes in the

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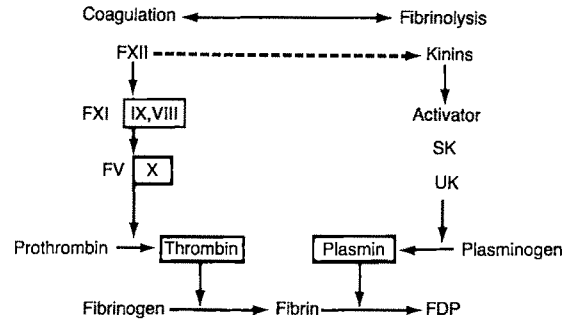


Fig. 1. Normal dynamic equilibrium between coagulation and fibrinolysis. *FXII,* Factor XII; *FXI,* factor XI; *FV,* factor V; *FDP,* fibrin degradation products; *SK,* streptokinase; *UK,* urokinase.

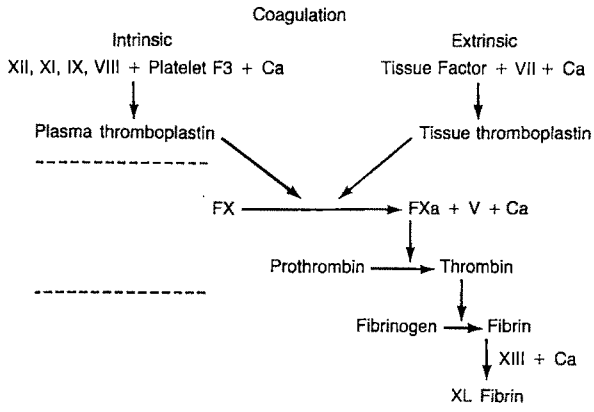


Fig. 2. Intrinsic and extrinsic coagulation pathways.

Table I. Royal College of General Practitioners data on mortality from vascular complications in OC users

Deaths			*Relative risk*		*Attributable risk*	
Current user	*Past user*	*Never user*	*Current versus never*	*Past versus never*	*Current versus never*	*Past versus never*
29	31	7	4	4	22	24

Number of deaths and attributable risk are per 100,000 per year.

Table II. Venous thromboembolic disease

Type	*Incidence (cases/10,000 women/yr)*		*Relative risk*	*Attributable risk (cases/10,000 women/yr)*
	Current users	*Nonusers*		
Superficial leg vein thrombosis	19	8	2	11
Deep vein thrombosis or pulmonary embolism	11	3	4	8
Postoperative venous thrombosis	61	30	2	31

rate of venous flow). The risk of overt venous thromboembolism appears to be related primarily to the estrogen component of OCs and is confined to the time of current usage.

The pathogenesis of arterial vascular complications—myocardial infarction and stroke—associated with OC use appears to be much more complex than that responsible for venous complications and relates to both increased platelet activity and fibrin deposition and to other factors that contribute to accelerated atherogenesis. These factors include increased arterial blood pressure and changes in glucose tolerance and lipoprotein and cholesterol metabolism. Once an atherogenic lesion develops, the process is difficult to reverse because of built-in self-maintaining or autoregulating mechanisms that are involved in this arterial lesion. The progestogen component, which was formerly regarded as the "innocent" party of the combined OC, is now considered to be an important factor with respect to arterial vascular disorders.

Blood coagulation and thrombosis

Blood coagulation and thrombosis are not identical processes, but they are intimately related. Vascular thrombosis can be regarded as hemostasis involving platelets and blood clotting occurring in the wrong place and involving an intact blood vessel. When new types of OCs or estrogen-containing drugs are introduced, it is important to investigate their effects on the physiologic processes involved in hemostasis.

The blood coagulation and fibrinolytic systems are normally in a dynamic equilibrium that controls the formation and removal of fibrin. Both systems are dependent on proteolytic enzymes, which are produced from inactive precursors: With blood coagulation, thrombin is derived from prothrombin, and in the fi-

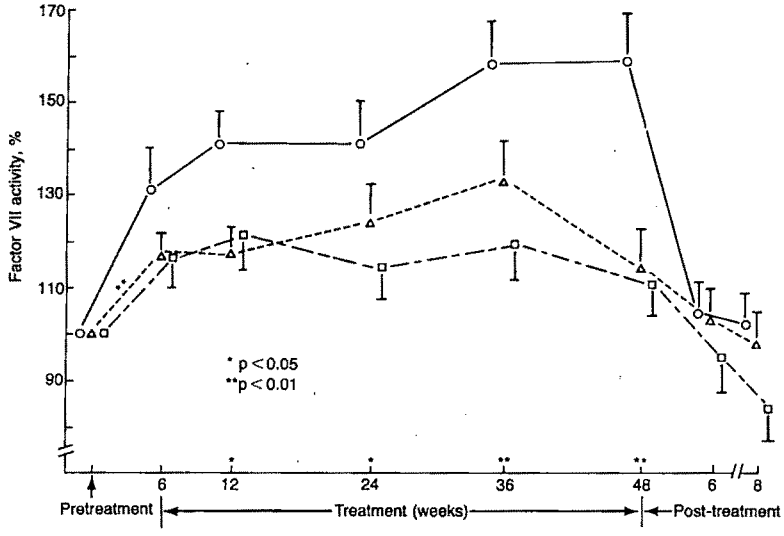


Fig. 3. Effects of oral contraceptives containing 50 and 30 μg estrogen on coagulation factor VII. Norethindrone acetate plus 50 μg estrogen, o——o; norethindrone acetate plus 30 μg estrogen, Δ - - - - Δ; levonorgestrel plus 30 μg estrogen, □ — — — □.

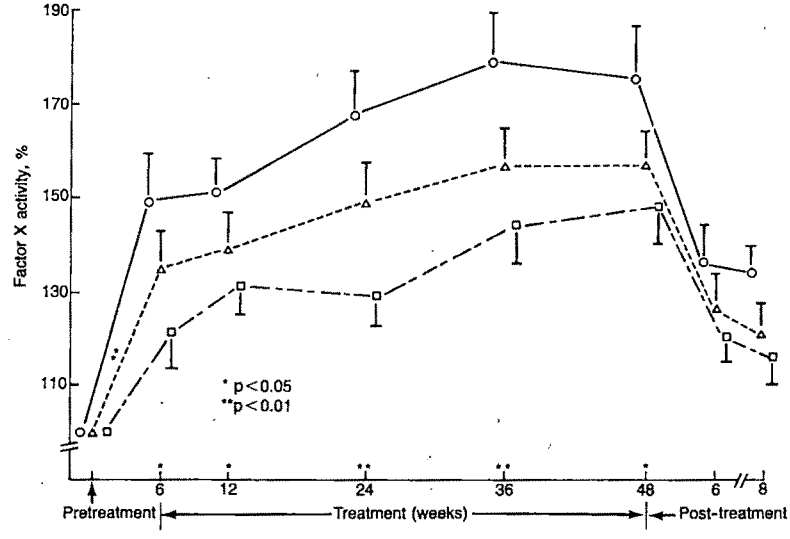


Fig. 4. Effect of oral contraceptives containing 50 and 30 μg estrogen on coagulation factor X. Norethindrone acetate plus 50 μg estrogen, o——o; norethindrone acetate plus 30 μg estrogen, Δ - - - - Δ; levonorgestrel plus 30 μg estrogen, □ — — — □.

Table III. Effect of 50 and 30 μg estrogen OCs on the hemostatic system

Formulation	*No. of patients*
Norethindrone acetate: 1.0 mg norethindrone acetate and 50 μg mestranol	31
Norethindrone acetate: 1.5 mg norethindrone acetate and 30 μg ethinyl estradiol	27
Levonorgestrel: 150 μg levonorgestrel and 30 μg ethinyl estradiol	28

brinolytic system plasmin is derived from plasminogen (Fig. 1).

Blood coagulation and fibrinolysis also share a common activation from factor XII. The coagulation system is designed as a biochemical amplifier, and the initial trigger may emanate from within the blood itself (the intrinsic system) or from outside the blood vessel from the tissues (the extrinsic system). The interaction of coagulation factors leads to the formation of either plasma thromboplastin or tissue thromboplastin, which

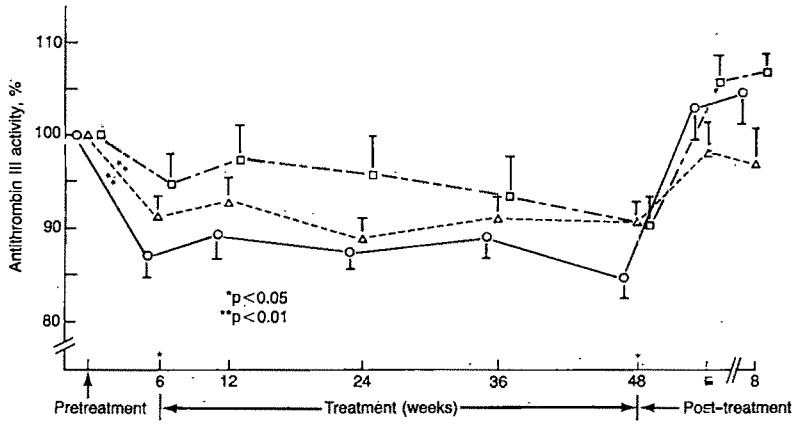


Fig. 5. Effect of oral contraceptives containing 50 and 30 μg estrogen on antithrombin III. Norethindrone acetate plus 50 μg estrogen, o——o; norethindrone acetate plus 30 μg estrogen, Δ - - - - Δ; levonorgestrel plus 30 μg estrogen, □ — — — □.

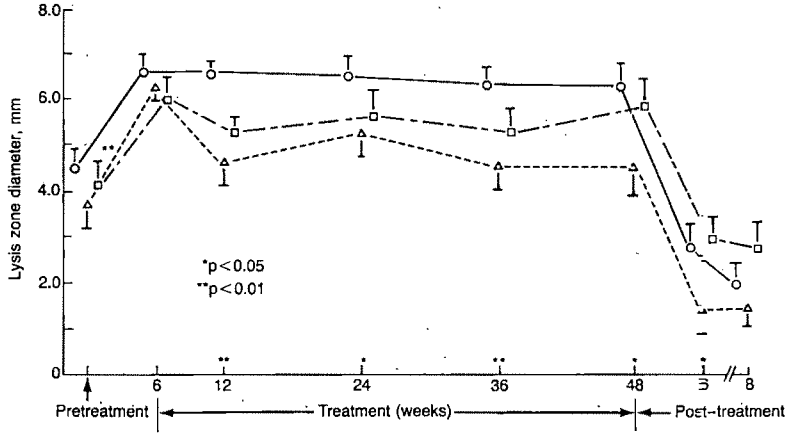


Fig. 6. Effect of oral contraceptives containing 50 and 30 μg estrogen on the fibrinolytic system (fibrin plate assay [resting]). Norethindrone acetate plus 50 μg estrogen, o——o; norethindrone acetate plus 30 μg estrogen, Δ - - - - Δ; levonorgestrel plus 30 μg estrogen, □ — — — □.

converts factor X to its activated form. The complex of activated factor X, with factor V and calcium converts prothrombin to thrombin. Thrombin converts soluble fibrinogen into fibrin, which in the presence of factor XIII and calcium forms cross-linked fibrin (Fig. 2).

Estrogen therapy is known to influence the clotting mechanism by increasing the levels of factors II, VII, VIII, IX, and X, fibrinogen, and soluble fibrin. Estrogen also decreases the levels of antithrombin III and vessel wall fibrinolytic activator. The effects of OCs on blood coagulation have been recently reviewed by Beller and Ebert[5] and Notelovitz.[6]

Comparative studies of OCs

The comparative effects of different low-dose estrogen/progestogen contraceptives on the hemostatic system are best investigated by serial studies in healthy women. We have carried out two major studies, with the following objectives: (1) to compare the effects of 50 and 30 μg estrogen OCs on blood coagulation, fibrinolysis, and platelets; (2) to compare the effects of 30 μg ethinyl estradiol combined with different progestogens.

Comparison of 50 μg versus 30 μg estrogen. Participants in this study received either 50 μg of mestranol combined with 1.0 mg norethindrone acetate, 30 μg of ethinyl estradiol in combination with 1.5 mg of norethindrone acetate, or 30 μg of ethinyl estradiol combined with 150 μg of levonorgestrel[7] (Table III). Blood samples were taken for laboratory assay during a pretreatment cycle, after 6 12, 24, 36, and 48 weeks of treatment, and at 6 and 8 weeks after cessation of treatment.

Factor VII levels increased sharply with both estro-

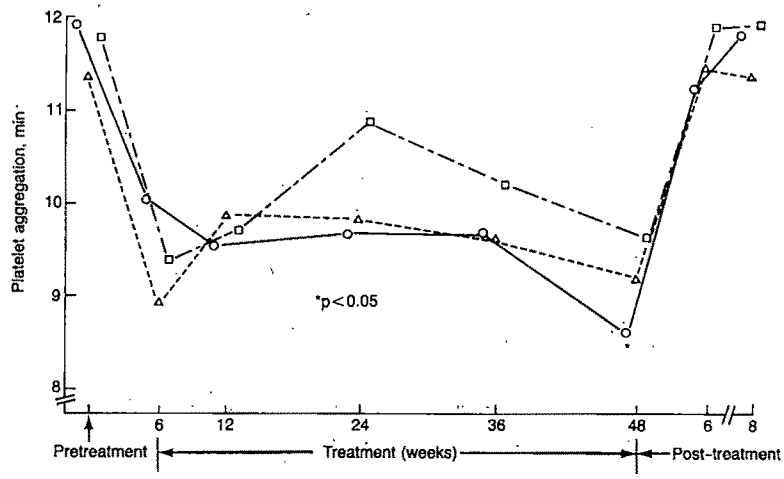


Fig. 7. Effect of oral contraceptives containing 50 and 30 μg estrogen on platelet aggregation (Chandler's tube method). Norethindrone acetate plus 50 μg estrogen, o——o; norethindrone acetate plus 30 μg estrogen, Δ - - - - Δ; levonorgestrel plus 30 μg estrogen, □ — — — □.

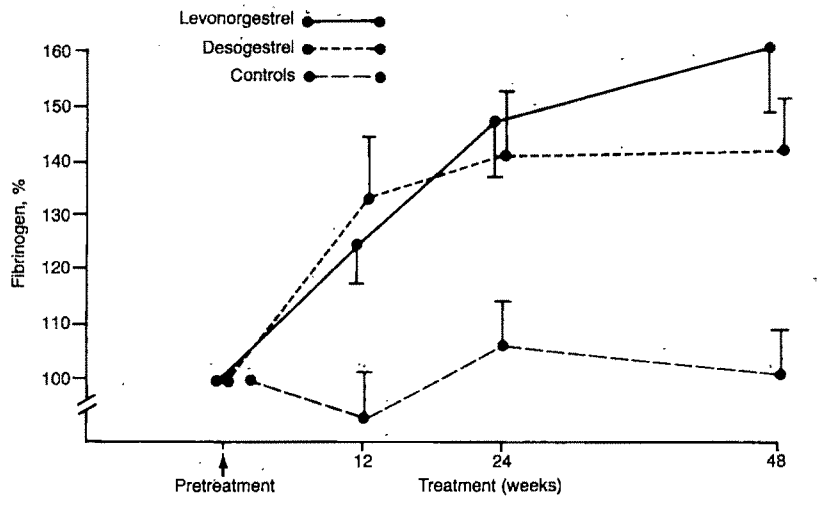


Fig. 8. Effects of levonorgestrel triphasic and desogestrel monophasic on fibrinogen.

Table IV. Comparative study of triphasic levonorgestrel and monophasic desogestrel

Formulation	*No. of patients*	*1 yr*
Levonorgestrel: 50-125 μg levonorgestrel combined with 30-40 μg ethinyl estradiol (triphasic formulation)	30	20
Desogestrel: 150 μg desogestrel and 30 μg ethinyl estradiol	36	13

gen dosages, but the increase with the 50 μg OC was significantly higher than that occurring with the two 30 μg preparations (Fig. 3). Similar results were noted with factor X. The least increase in factor X activity occurred with the levonorgestrel combination (Fig. 4). Levels of the coagulation inhibitor antithrombin III decreased significantly in patients who received 50 μg OCs and to a lesser extent with the 30 μg OCs that contained norethindrone acetate. In the group who received the 30 μg OC that contained levonorgestrel, antithrombin III levels did not significantly decrease until the forty-eighth week (Fig. 5).

The resting plasma fibrinolytic activity was significantly increased with 50 and 30 μg preparations, and the greatest increase was found with the 50 μg OC (Fig. 6). Fibrinolytic activity after venous occlusion also increased significantly with all three preparations.

Spontaneous platelet aggregation with the Chandler tube method showed that both the 50 and 30 μg formulations produced significant shortening of the ag-

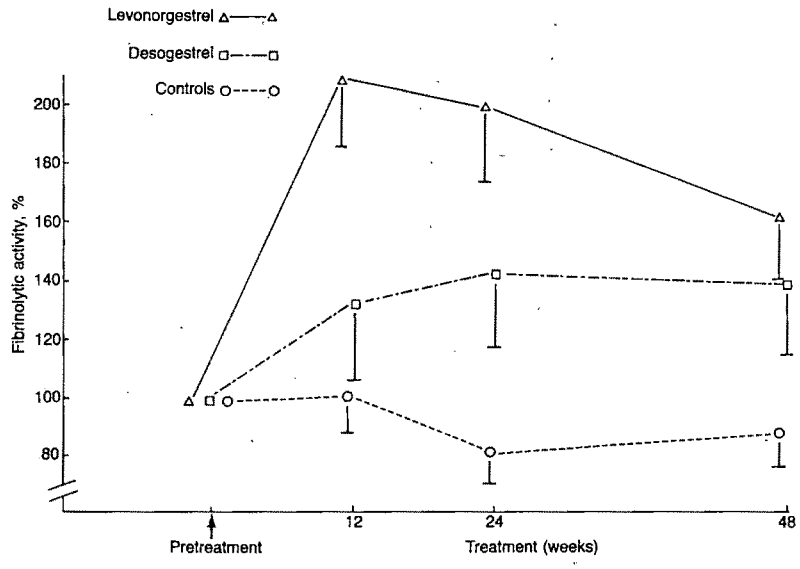


Fig. 9. Effects of levonorgestrel triphasic and desogestrel monophasic on fibrinolytic activity.

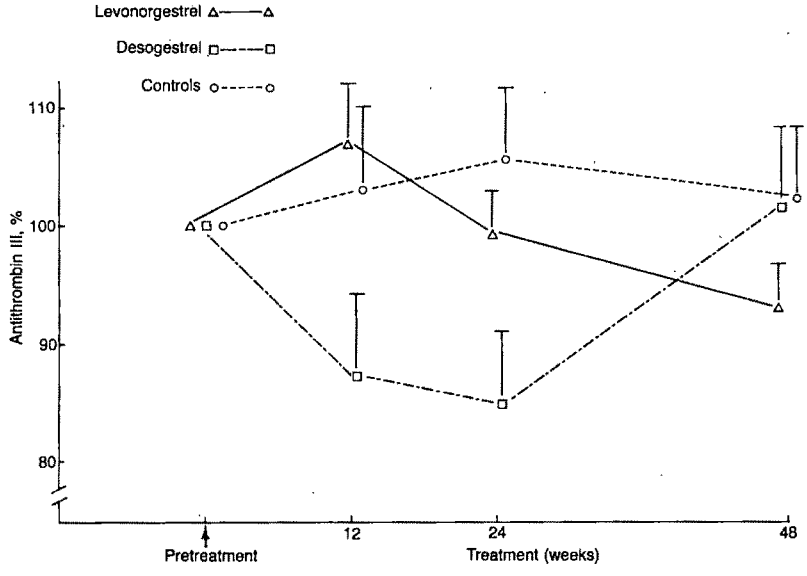


Fig. 10. Effects of levonorgestrel triphasic and desogestrel monophasic on antithrombin III (immunologic method).

gregation time, indicating enhanced platelet activity (Fig. 7).

These studies show that: (1) The effects of ethinyl estradiol on coagulation, fibrinolysis, and platelet function are related to dose, with 50 μg producing significantly greater changes than 30 μg; (2) the effects of 30 μg of ethinyl estradiol are modified by the progestogen that is used in combination.

Effects of different progestogens. Recently, we have compared the effects of triphasic levonorgestrel with monophasic desogestrel (Table IV). The effects of both combinations on fibrinogen are shown in Fig. 8. Fibrinolytic activity increased less with desogestrel than with triphasic levonorgestrel (Fig. 9). We also found that antithrombin III levels remained unchanged with triphasic levonorgestrel and decreased with desogestrel at 12 and 24 weeks on treatment but not at 48 weeks (Fig. 10).

In summary, these studies, which have compared 50 μg estrogen contraceptives with 30 μg estrogen contraceptives and different progestogens, show clearly that the changes in coagulation activity and fibrinolysis are related to the dose of estrogen and progestogen. The progestogen that is used in combination with low-

dose estrogen may potentiate or reduce the estrogen effects. The low-dose OC formulations in current use produce significantly less change in the hemostatic system than do preparations that contain 50 μg of estrogen. The estrogen/progestogen combinations also increase fibrinolytic activity, which most probably balances the increase in coagulation activity, thus preserving the dynamic equilibrium between coagulation and fibrinolysis.

Current research is aimed at finding low-dose formulations of estrogen/progestogen that will produce the least change in the hemostatic system and in other metabolic processes. This appears to be the most logical way of minimizing the risk of vascular complications and thus increasing the safety of oral contraception for the millions of women who use this method of fertility control.

I would like to thank Dr. Ali Sabra, Leisha Daly, and Eilis Carroll for their excellent teamwork in these research studies.

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Progestogen potency in oral contraceptive pills

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The most important target organs for combined oral contraceptive preparations are the anterior pituitary gland and the uterus. The long-term unopposed administration of estrogen produces endometrial hyperplasia and amenorrhea, which are unacceptable to most women and their medical advisors. Cyclic administration of progestogens in combination with the estrogen, however, produces predictable endometrial shedding and achieves a regular and acceptable bleeding pattern in most women. This was the main reason that the "delay of menses" test was adopted as the earliest clinical means of comparing the relative potencies of progestogens that were administered orally. Recently attempts have been made to compare the potency of progestogens on the other organ systems by the extrapolation of data derived from studies on the endometrium. This is inappropriate, inasmuch as the effects of progestogens and estrogens independently and in combination differ greatly depending on the target organ. In this article, the literature on this controversial subject is reviewed. (Am J Obstet Gynecol 1987;157:1048-52.)

Key words: Oral contraceptives, progestogen potency, delay of menses test, lipid metabolism

To appreciate fully the confusion that currently surrounds the concept of the potency of progestogen, it is necessary to know the early history of the combined oral contraceptive (OC).

History of the combination pill

The first 19-norprogestogen, norethisterone, was synthesized in October 1952, and the U.S. patent for the drug was filed a month later by Carl Djerassi on behalf of Syntex. In August 1953, nearly a year later, Frank B. Colton of Searle filed the patent for norethynodrel. Although it was known that norethynodrel was converted to norethisterone by weak acids and that this conversion almost certainly occurred in the stomach after ingestion, commercial reasons led to the choice of norethynodrel as the preparation that was used in the first studies of the OC pill. According to Vaughan,[1] the reason given for this decision was that norethisterone had been shown to produce testicular enlargement in rats, and it was feared that this compound might have masculinizing effects. However, Pincus, who played a

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major part in the early studies on the pill, was closely associated with Searle, and it is not altogether surprising that he and his associates favored the Searle compound in clinical trials. Thus even the very earliest stages in the development of the OC pill were marked by confusion about the relative actions of its progestogen components.

Further confusion followed the discovery that norethynodrel contained an estrogen contaminant, mestranol. When this contaminant was eliminated, cycle control, which had previously been satisfactory, became unpredictable and breakthrough bleeding occurred. The estrogen was therefore reintroduced in an amount that was equivalent to 1.5% of the progestogen dose. Thus it is apparent that the resulting "combination pill" Enovid, which contained 10 mg of norethynodrel and 150 μg of mestranol, was essentially a product of chance. This early experience with the pill clearly demonstrated an interaction between the estrogen and the progestogen components on the endometrium, an interaction that has frequently been ignored when progestogens such as levonorgestrel and desogestrel have been compared with the earlier products that were derived from norethisterone.

Emergence of potency as an issue

Until recently, the relative potency or bioactivity of the constituents of OCs had been of little interest to clinicians. In 1983 the publication by Pike et al.[2] of data that linked the risk of breast cancer to the progestogen content of the pill, and in particular the "potency" of the progestogens in different preparations, has, however, focused attention on this issue.

Of the innumerable target organs for the steroids in combination OC preparations, the anterior pituitary gland and the uterus are clinically the most important. The initial doses of estrogen and progestogen "chosen" for the pill appeared to inhibit ovulation effectively, but in practice it was difficult to assess "potency" by comparing the effects of different progestogens on the ovulatory process. In addition, it soon became apparent from the early clinical trials that irregular bleeding was unacceptable to women taking the pill and that this effect on the endometrium was likely to be as important a factor to the success of the pill as its ability to prevent pregnancy.

The delay of menses test

The endometrial effects of the pill were relatively easy to measure in clinical practice; therefore it was logical that the earliest efforts to compare the potency of progestogen in women relied on the so-called delay of menses test. This originated from an observation by Greenblatt et al.[3] that if women with normal menstrual cycles were given a progestogen, but not an estrogen, after they had ovulated, menstruation could be postponed. On the basis of these observations, Swyer et al.[4] developed an assay system in which they administered the test dose of progestogen for 20 days beginning on the twentieth day of the cycle. If "menstruation" was postponed to the fortieth day, the result was positive; if not, it was negative. Adjustment of the dose enabled a multipoint assay from which the median effective dose could be established to enable the comparison of the relative potencies of different progestogens.

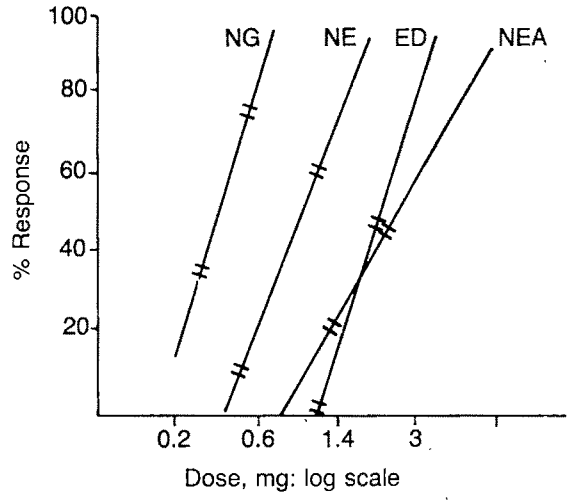


Fig. 1. Dose-response lines in the delay of menses test to show parallelism. *NG*, Norgestrel; *NE*, norethisterone; *ED*, ethynodiol diacetate; *NEA*, norethisterone acetate. Constant estrogen dose of 50 μg.

Table I. Activity of progestogens in the delay of menses test[15]

Progestogen	*Greenblatt*	*Swyer*
Norethisterone	1	1
Norethisterone acetate	2	0.4
Ethynodiol diacetate	15	<1
Norgestrel	30	2.5

The application of this test to different progestogens has given widely differing results as shown in the extensively quoted studies of Greenblatt and Swyer (Table I). These results have been criticized for several reasons.[5-7] First, the biologic activity of the various progestogens differs greatly depending on whether the women receiving them have been treated with estrogen. Second, there have been variations in the amount of estrogen that is combined with the progestogen in the study. Third, the published comparisons have seldom been based on parallel dose-response curves. Although the data published by Greenblatt[8] do not appear to be standardized, those of Swyer largely satisfy the above criticisms. The most recent studies of Swyer[9, 10] provide the only data available on the delay of menses test in

Table II. Plasma high-density lipoprotein cholesterol in women receiving combination oral contraceptive preparations that contain 30 to 35 μg of ethinyl estradiol and varying doses of levonorgestrel, norethisterone, or ethynodiol diacetate

Progestogen	*Dose (μg)*	*High-density lipoprotein cholesterol (mmol/L) (mean ± SD)*
Levonorgestrel (plus 30 μg ethinyl estradiol) (n = 20)	50-75-125	1.53 ± 0.36
	150	1.50 ± 0.19
	250	1.38 ± 0.19
Norethisterone (plus 35 μg ethinyl estradiol) (n = 20)	500	1.61 ± 0.22
	1,000	1.48 ± 0.20
Ethynodiol diacetate (plus 30 μg ethinyl estradiol) (n = 20)	2,000	1.39 ± 0.20
Controls (n = 20)	Nonpregnant	1.57 ± 0.26
	Pregnant	1.65 ± 0.35

Table III. Plasma high-density lipoprotein cholesterol in women receiving triphasic preparations containing levonorgestrel or gestodene

	High-density lipoprotein cholesterol (mmol/L) (mean ± SD)	
Progestogen	*Before therapy*	*Sixth month*
Levonorgestrel (n = 18)	1.50 ± 0.33	1.54 ± 0.34
Gestodene (n = 16)	1.50 ± 0.25	1.51 ± 0.25

women treated with combination OC preparations that contain 50 μg of ethinyl estradiol (Fig. 1). The relative potency or bioactivity of the progestogens included in this study is in keeping not only with the recent evidence that norethynodrel and ethynodiol diacetate must be converted to norethisterone to become bioactive but also with the available data on endometrial subnuclear vacuolation produced by these progestogens.[11, 12] These data would suggest that the relative bioactivity on the endometrium of norethisterone (and the progestogens derived from this compound), when combined with 50 μg of ethinyl estradiol, is one fifth that of levonorgestrel in combination with the same amount of estrogen. It is important to be aware that no comparable data exist for preparations that contain 30 to 35 μg of ethinyl estradiol or preparations that contain the newer progestogens such as desogestrel, gestodene, or norgestimate.

Effect of progestogens on lipid metabolism

Interpretation of the biologic activity of progestogens was further complicated by the recognition that no synthetic progestogen has pure progestogenic activity. The chemical origin of the 19-norprogestogens from androgens confers weak androgenic (or antiestrogenic) actions, and the conversion of norethisterone-derived compounds to estrogen may paradoxically produce estrogenic actions.[13] Whereas estrogens and progestogens act synergistically on the endometrium, their effects on certain metabolic processes are antagonistic. This is especially relevant to lipoprotein metabolism. The data of Wahl et al.[14] on the relative effects of the older high-dose pills demonstrated that these pills had an essentially estrogen-dominant effect, as judged by the significant increases in total triglycerides and the low-density lipoprotein cholesterol that they produced. On the other hand, the high-density lipoprotein cholesterol concentrations rose in women using norethisterone-derived progestogens and fell in women who received a high dose of norgestrel. These observations highlight the virtual impossibility of meaningful comparisons of progestogen potency in relation to lipoprotein metabolism unless the doses of both the estrogen and the progestogen in the OCs being studied are taken into account.

In the last decade, efforts have been made to minimize the potentially atherogenic reduction in the high-density lipoprotein cholesterol that was observed with some of the relatively progestogen-dominant pills that were used in the 1970s because of fears that these changes might account for the significant increase in cardiovascular disease mortality rates reported in epidemiologic studies of pill users.[15] Recent data collected in Oxford and elsewhere on modern low-dose combination OC preparations suggest that this aim has largely been achieved (Tables II and III).

Progestogens and the breast

Limited studies that are available on the breast suggest that whereas progestogens inhibit endometrial proliferation, natural progesterone has the opposite effect on breast lobule epithelium[16] (Fig. 2). Therefore the relative bioactivity observed in studies of proges-

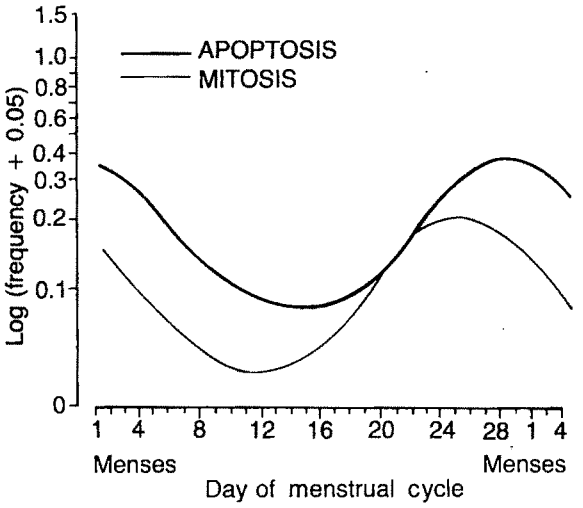


Fig. 2. Mitosis and apoptosis in the menstrual cycle.

togens on the endometrium cannot be extrapolated to the breast. Although studies are in progress to clarify the effect of the pill on this target organ (Anderson TJ, personal communication), their results are not yet available.

The future

This brief review cannot examine the many effects of OCs on end-organs, but certain general points can be made. New combinations of estrogen and progestogen must be "designed" not only to be highly effective in inhibiting ovulation and altering cervical mucus and endometrium but also in achieving optimal cycle control with minimal metabolic perturbation. This involves careful study of as many measurable end points as possible, including lipid and carbohydrate metabolism and coagulation processes. It will never be possible to assess all effects on end-organs and some chances will have to be taken, as they were with Enovid.

Several questions remain to be answered. For example, do the increases in high-density lipoprotein cholesterol (in particular the high-density lipoprotein 2 subfraction) that have been observed with recent formulations protect the patient from atherogenic disease? Does it matter that most of these preparations continue to produce significant alterations in coagulation factors[17] and other proteins such as sex hormone–binding globulin?[18] Do minor alterations in metabolic processes matter if values remain within the normal range? Are the biologic effects of OCs related to the highly variable circulating concentrations of the individual components that have been observed[7,19-21] (Figs. 3 and 4), and, if so, should efforts be made to tailor the "pill dose" to the needs of each person? This would presumably mean that it would be more appropriate for those

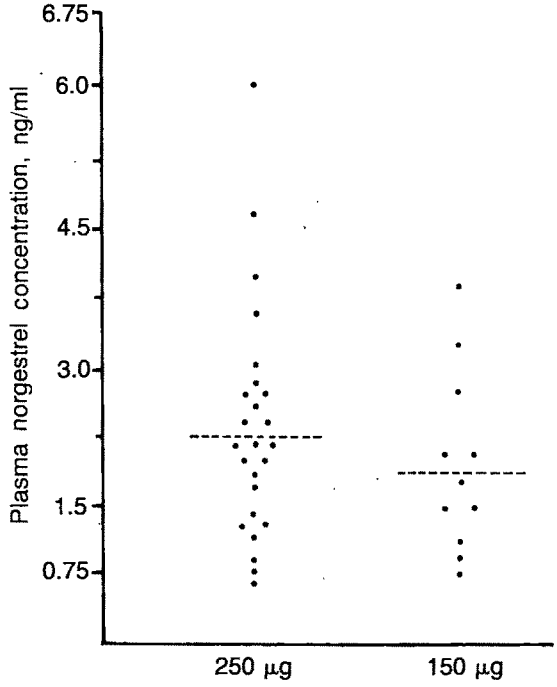


Fig. 3. Plasma norgestrel concentrations in women receiving long-term treatment with combined OCs containing 30 μg of ethinyl estradiol and either 150 or 250 μg of levonorgestrel. (Samples taken 12 hours after ingestion.)

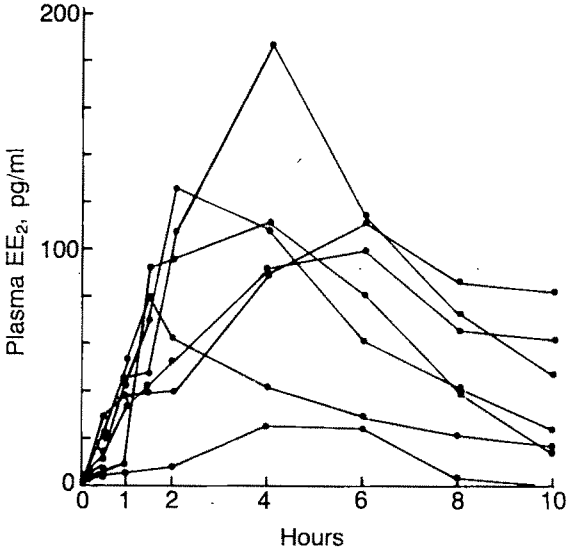


Fig. 4. Individual variation in plasma levels of ethinyl estradiol *(EE_2)* given under rigidly standardized conditions.

women who experience poor cycle control while taking low-dose preparations to take the older high-dose preparations. It will take many years to answer these questions and until then we will have to accept our very incomplete understanding of "progestogen potency" and the pill.

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Phasic approach to oral contraceptives

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Oral contraceptives were initially very high in estrogen and progestogen and had a uniform dosage schedule throughout the entire cycle. These early oral contraceptives were associated with high rates of side effects and complications. As a result, the hormone levels have been gradually reduced to attain the minimal amount necessary for contraception while most of the undesired side effects and complications have been eliminated. Recently, a new approach has been added. This mechanism consists of altering the dose of estrogen or progestogen or both during the month and is referred to as the phasic approach. The newest phasic pills now use a triphasic approach. Three basic formulations of the triphasic pill have been developed. In two of the three formulations, the progestogen level is altered, whereas the estrogen level remains stable; in the third formulation, both estrogen and progestogen are changed. Multiple clinical studies of the triphasics, such as ethinyl estradiol and levonorgestrel, prove that there is a significant reduction in the incidence of side effects and complications related to oral contraception. (Am J Obstet Gynecol 1987;157:1052-8.)

Key words: Oral contraceptives, triphasics, reduction of side effects

Ovulation is a complex interaction of multiple neuroendocrine and hormonal events (Fig. 1).[1] Interruption of this relationship results in anovulation and infertility.[2] If the interruption is voluntary, its mechanism should be as free as possible from undesirable side effects and complications.

From the Department of Obstetrics and Gynecology, John A. Burns School of Medicine, Kapiolani-Children's Medical Center.

In the early and mid-1950s, researchers developed steroidal medications that were effective in interrupting these ovulation pathways and producing a state of anovulation and temporary infertility. These medications were first marketed in the early 1960s and used a 19-nortestosterone compound with a synthetic estrogen. The original formulation contained 9.85 mg of norethynodrel acetate and 0.150 mg of mestranol. How-

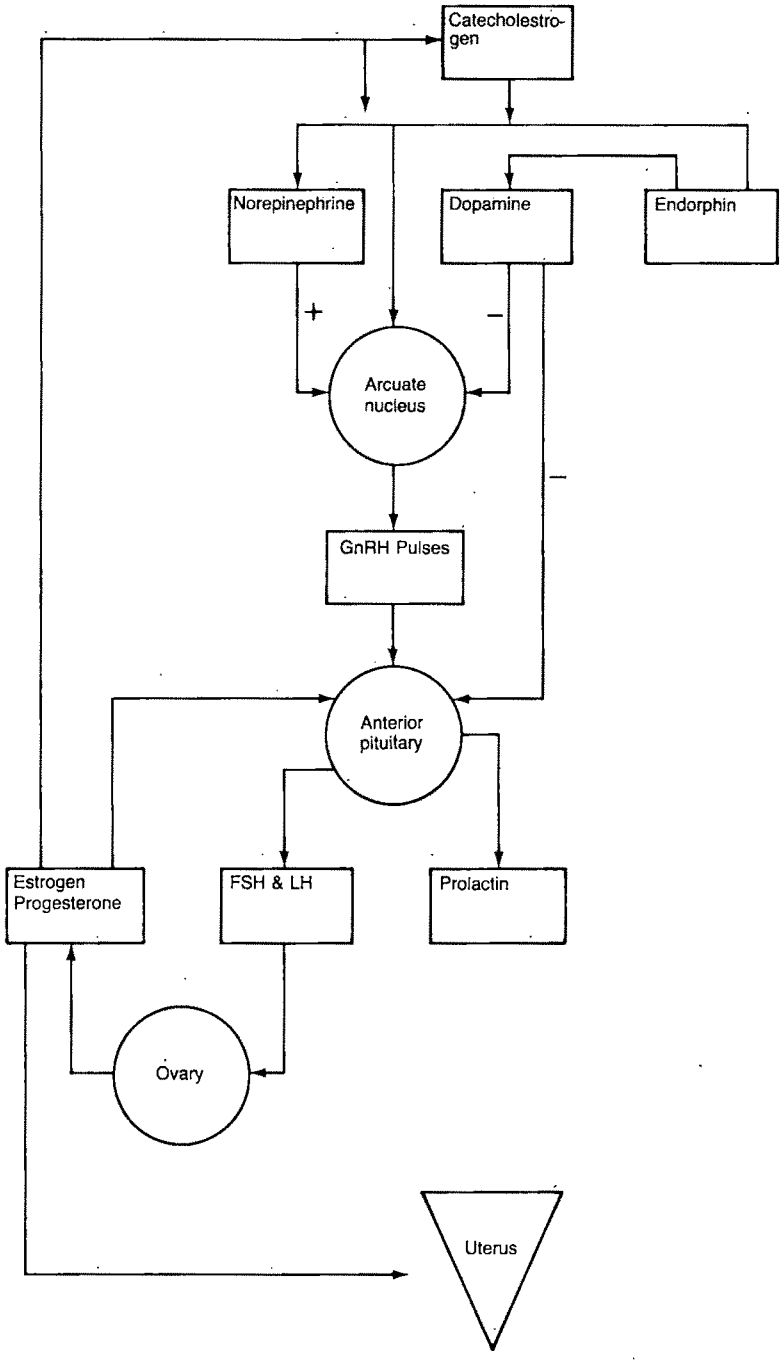


Fig. 1. Neuroendocrine control. (Reprinted with modification from Speroff L, Glass RH, Case NG. Clinical gynecological endocrinology and infertility. 3rd ed. Baltimore: Williams & Wilkins, 1983:56.) (*GnRH* = Gonadotropin-releasing hormone; *FSH* = follicle-stimulating hormone; *LH* = luteinizing hormone.)

ever, a short time later, reports of undesirable side effects and complications began to appear. By the late 1960s and early 1970s, there were multiple reports of serious complications including stroke, thromboembolism, and hypertension.[3-7]

In an attempt to reduce these adverse effects, the manufacturers reduced the steroid content and introduced new progestogens. Except for a brief trial with a sequential approach and the currently available minipill, all of the formulations have used a combined daily dose of progestogen and estrogen. Through the 1970s and 1980s, reduction of steroid content has continued, until now the preferred combination pill contains 1 mg or less of a progestogen and 30 to 35 μg of an estrogen.

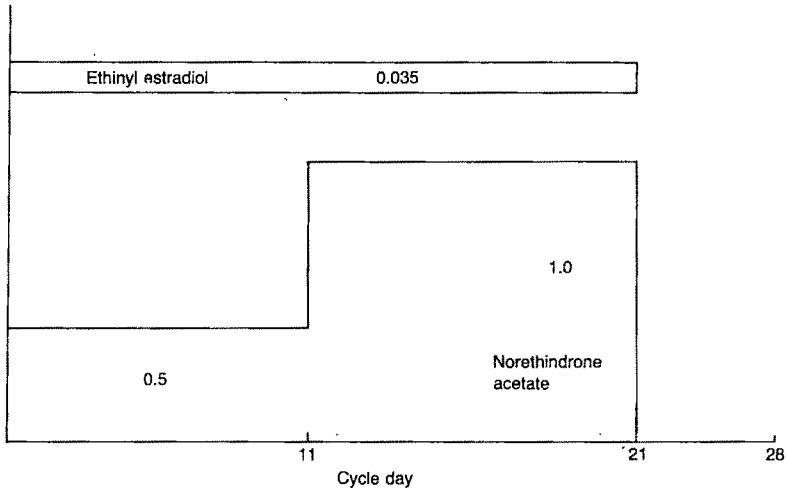


Fig. 2. Biphasic oral contraception.

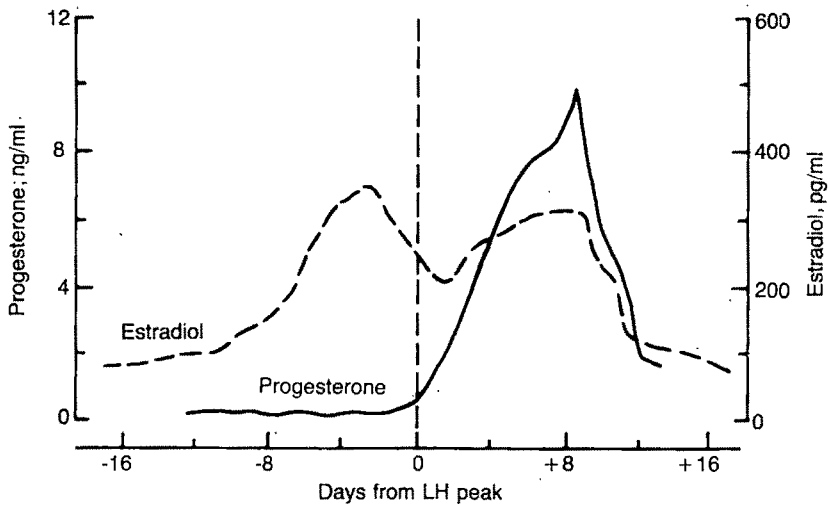


Fig. 3. Normal hormonal cycle.

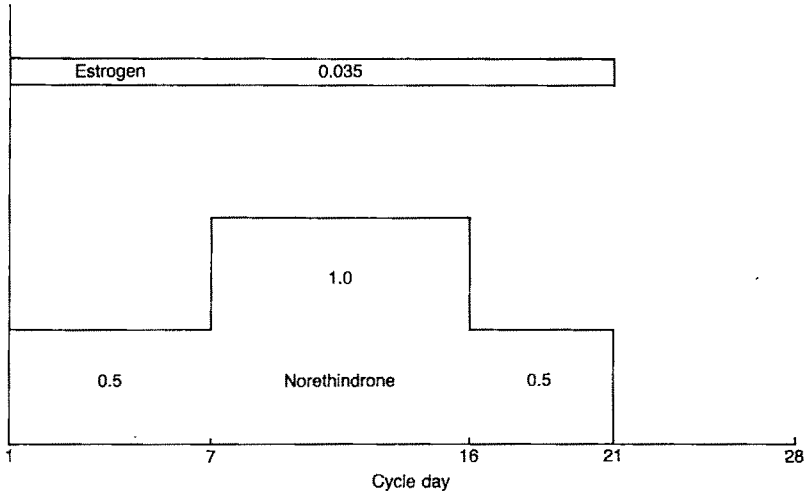


Fig. 4. Triphasic No. 1.

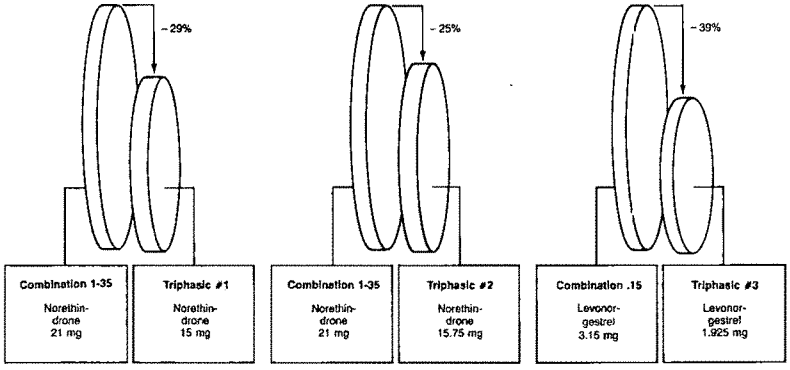


Fig. 5. Progestogen content compared with combination pill.

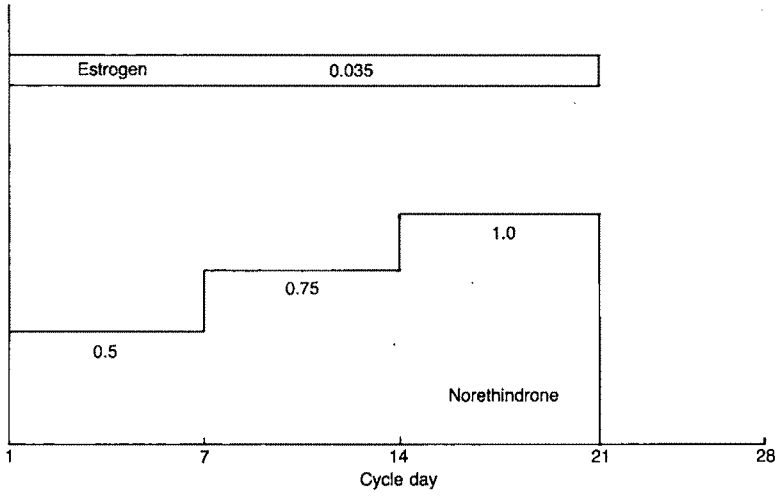


Fig. 6. Triphasic No. 2.

It is widely believed that these low-dose formulations have far fewer side effects and complications than do the earlier higher-dose pills.[8-10]

Recently, newer formulas have been introduced that attempt to lower the hormonal content of the average cycle even further. Although there is insufficient evidence to prove that the total steroid content of any given cycle is as important as the daily amount, it seems logical that any method that lowers the total amount of steroid ingested would be a positive step toward further reduction of complications and side effects.

The first of these newer pills was a biphasic pill. The patient took one fixed dose for the first 10 days of the cycle and another dose for the next 11 days (Fig. 2). This pill used two existing formulas and resembled the earlier sequential pill. However, this formulation had a high percentage of side effects such as breakthrough bleeding and offered very little to patients.

The next step forward was the development of a triphasic pill. In this formulation, the patient receives a different dosage schedule in each of three separate periods within the same cycle. I will discuss this type of approach to oral contraception in this paper.

Normal cycle control

To understand fully the principles of the phasic approach, it is necessary that we first review the hormonal pattern of the normal cycle. In this way, we can compare the natural with the controlled cycle (Fig. 3).

The initial event in any menstrual cycle is the menstruation that results from hormonal withdrawal, ischemia, and necrosis of the endometrium. At this phase of the cycle, estrogen production is at its lowest level. Estrogen gradually increases during the next 10 to 14 days and reaches a peak at the time of ovulation. This peak is followed by reduced production for the next 3 to 5 days. Estrogen levels then gradually rise and plateau until approximately day 24 or 25 when they drop to baseline. On the other hand, progesterone begins to rise 1 to 2 days before ovulation, reaches a peak

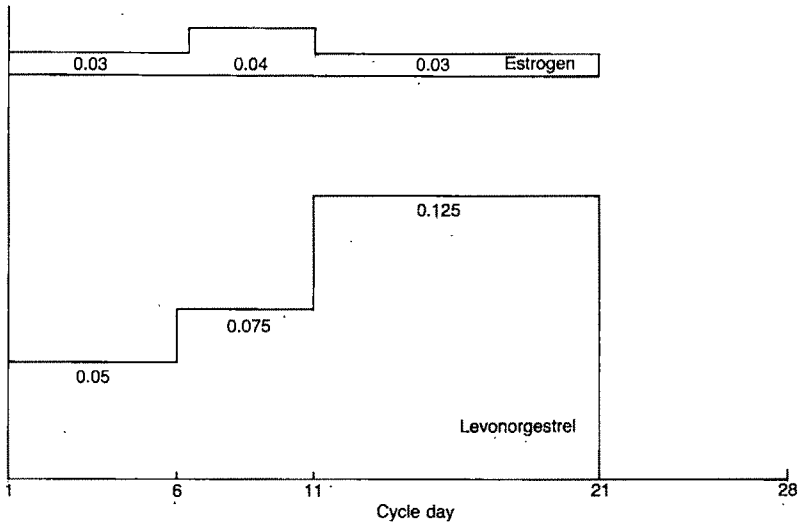


Fig. 7. Triphasic No. 3.

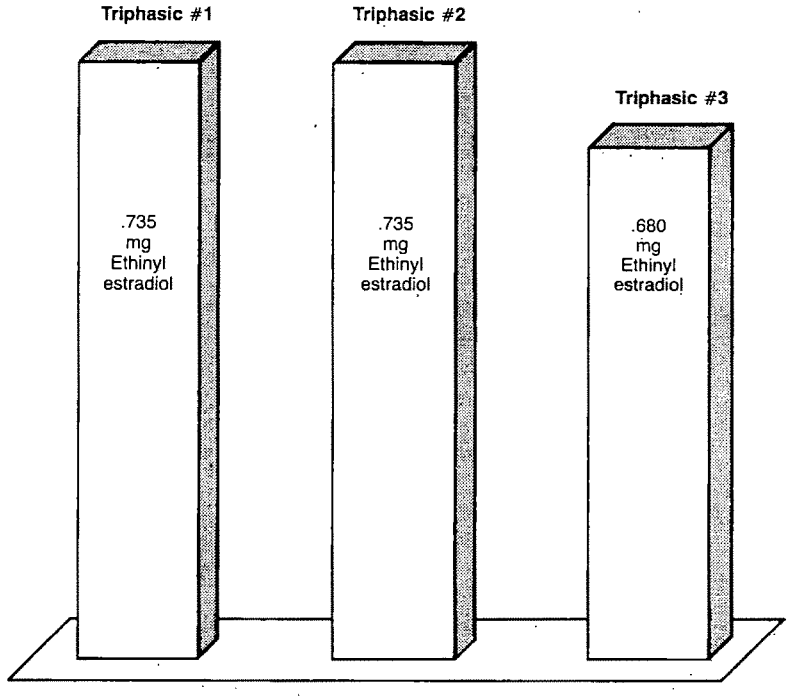


Fig. 8. Total estrogen content of triphasics.

on approximately day 19 or 20, and then begins to regress until it returns to baseline at day 28. The new triphasic pills attempt to imitate this cyclic rhythm.

Current preparations

Three triphasic formulations are marketed today. Each has been developed using a parent compound, currently marketed as an oral contraceptive, and then altering the hormonal content. In two of these three pills, the estrogen content, 0.035 mg of ethinyl estradiol, remains constant throughout the cycle whereas the progestogen content changes.

Fig. 4 shows how one of these formulations uses 0.5 mg of norethindrone for the first 7 days, followed by 9 days of 1 mg of norethindrone, and then returns to 5 days of 0.5 mg of norethindrone. The total steroid dosage is less in this formulation than in a 1/35 pill (Fig. 5), but the last 5-day reduction has led to early bleeding and difficulty with patient acceptance in my practice.

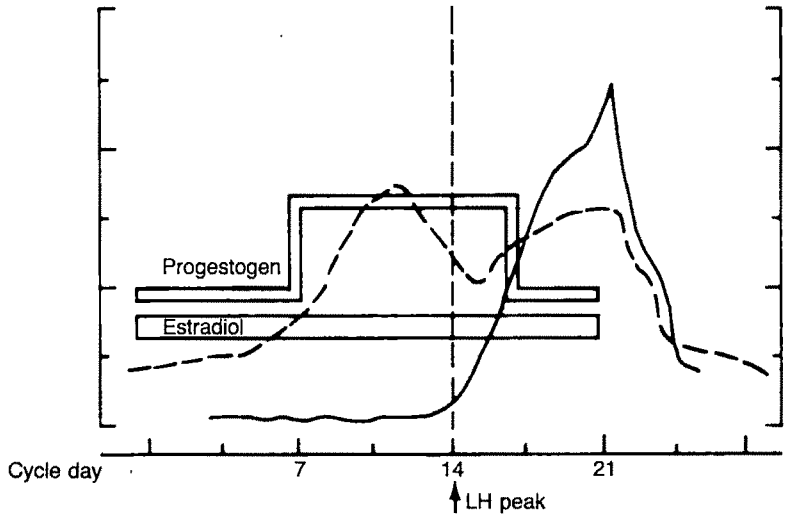


Fig. 9. Triphasic No. 1. (*LH* = Luteinizing hormone.)

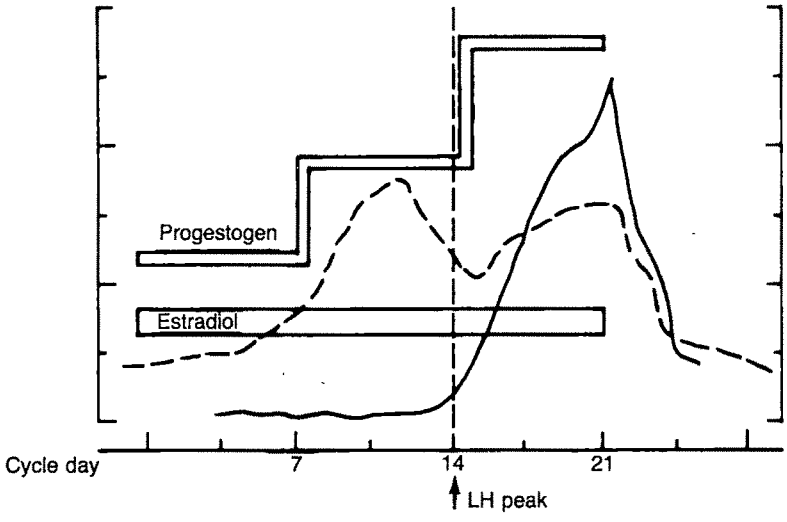


Fig. 10. Triphasic No. 2. (*LH* = Luteinizing hormone.)

A second formulation that uses the constant estrogen dose is seen in Fig. 6. This pill begins with 7 days of 0.5 mg of norethindrone, then increases to 0.75 mg of norethindrone for 7 days, and ends with 7 days of 1 mg of norethindrone. This formulation again gives a reduced hormone level compared with the typical 1/35 combination pill (Fig. 5).

The third triphasic preparation (Fig. 7) has a formulation different from the other two. Not only does this pill contain a different progestogen (levonorgestrel), it also alters the amount of estrogen and progestogen. In the first phase of this pill, the patient receives 0.03 mg of ethinyl estradiol and 0.05 mg of levonorgestrel for 6 days; for the next 5 days she receives 0.04 mg of ethinyl estradiol and 0.075 mg of levonorgestrel; during the last 10 days, the patient receives 0.03 mg of ethinyl estradiol again in combination with 0.125 mg of levonorgestrel. This formulation again reduces the total progestogen content of the cycle (Fig. 5). In addition, it also lowers the estrogen content compared with the other two triphasics (Fig. 8). The levonorgestrel triphasic is the only one of the three triphasic pills currently marketed that has been extensively studied as a triphasic.

The cycles of each triphasic are compared with the normal hormonal cycle in Figs. 9 through 11. Each of the three formulas has attempted to duplicate the normal cycle in a different fashion. Only one approaches the estrogen levels, and only two replicate the progesterone component.

In each of these formulations, the hormone content has been changed to give a reduced level of steroid.

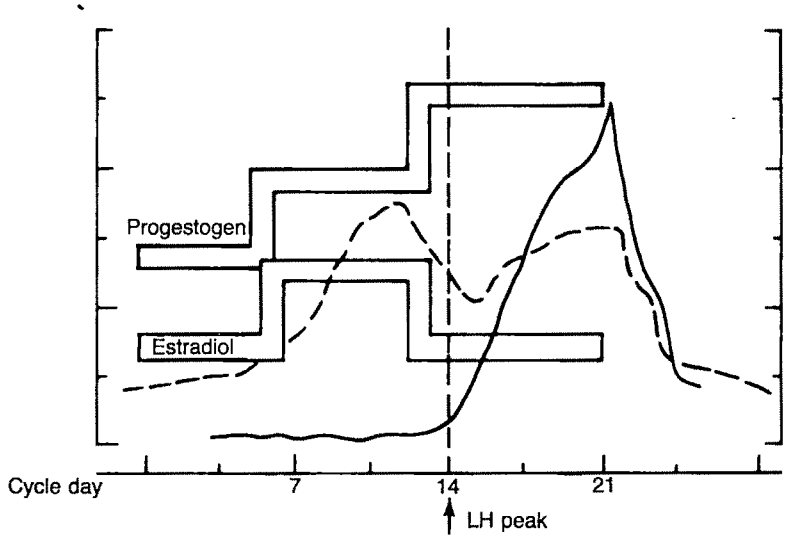


Fig. 11. Triphasic No. 3. (*LH* = Luteinizing hormone.)

Although the benefits of reduced total steroid content have yet to be proved, I believe that any attempt to reduce the total amount of steroid ingested during the cycle is potentially beneficial.[11] However, early reports show a slightly higher incidence of breakthrough bleeding than had been noted with the comparable fixed combination pill. This possibly reflects the reduced hormone level and indicates that we approach the lowest practical level with currently available steroids. On the other hand, early studies suggest that the levonorgestrel triphasic produces fewer alterations in metabolic pathways, lipid chemistry, coagulation profiles, and blood pressure. Other incidental side effects, such as acne, depression, and nausea, are also reduced.

In conclusion, the new phasic approach is an attempt to reduce the total steroid content and thereby reduce the negative impact of the "pill." It is also an attempt to emulate the normal menstrual cycle. For this reason, I believe that a triphasic pill of the physician's choice should be the initial agent for all patients desiring oral contraceptives.

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New progestogens in oral contraceptives

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The aim of using new synthetic progestogens (gestodene and norgestimate) in oral hormonal contraceptives is to find a combination that has a more beneficial effect on metabolism and endometrium than presently available formulations. Our studies with low-dose pills containing 30 μg ethinyl estradiol/150 μg levonorgestrel or 30 μg ethinyl estradiol/150 μg desogestrel compared with the new pills with 35 μg ethinyl estradiol/250 μg norgestimate or 30 μg ethinyl estradiol/75 μg gestodene revealed no significant alterations of serum glucose after glucose loading. With all four combination pills, insulin levels were slightly elevated when compared with controls. Studies of the lipid metabolism showed that depending on the type and estrogen combination, progestogens have different effects on lipid metabolism. The new progestogens seem to have a more pronounced effect on triglycerides, whereas total cholesterol and high-density lipoprotein cholesterol remain almost unchanged. In general, it could be shown that low-dose oral contraceptives have little impact on lipid metabolism. Studies with low-dose monophasic preparations, including the new formulations, reveal only a low effect on blood coagulation. According to our and other data on the new progestogens in oral contraceptives available so far, it can be expected that such low-dose monophasic and triphasic combination pills will be beneficial during longtime use with respect to side effects on the cardiovascular system and control of the menstrual cycle. (Am J Obstet Gynecol 1987;157:1059-63.)

Key words: Oral contraceptives, low-dose monophasic preparations, insulin levels, lipid metabolism, blood coagulation

The effects of the various progestogens in oral contraceptive pills have been studied extensively.[1, 2] Although a complete evaluation of the many different actions of the synthetic progestogens is impossible at the present time, this article will attempt to describe and compare the effects of known progestogens with those of the new synthetic progestogens.

Structural characteristics

All synthetic progestogens are derivatives of either 17α-hydroxyprogesterone or 19-nortestosterone. The 19-nortestosterone derivatives can be categorized according to structure into the estranes and the biologically more effective gonanes. The estranes are chemically characterized by the absence of a methyl group between ring A and B and by an ethinyl group in position 17α. These substitutions delay the compounds' inactivation in the liver and greatly reduce the androgenic effects. The gonanes share these structural features and are also characterized by an ethyl group in position 13. Among the gonanes are the most effective new synthetic progestogens, such as levonorgestrel, desogestrel, norgestimate, and gestodene, which are the focus of this paper.

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Table I. Main effects of synthetic progestogens in women

1. Inhibition of ovulation
2. Secretory transformation of estrogen-primed endometrium; inhibition of the proliferative process
3. Effect on tubal secretion and egg transport; inhibition of myometrial contractions
4. Delay of menstruation in estrogen-pretreated women
5. Changes in composition of cervical mucus
6. Increase in glycogen storage in the endometrium
7. Decrease of karyopyknotic index of the vaginal epithelium
8. Maintenance of pregnancy
9. Influence on hepatic metabolism
10. Increase in basal body temperature
11. Relative binding capacity to various steroid hormone receptors

Spectrum of biologic effects

The different progestogens are absorbed differently in the gastrointestinal tract. They are partially metabolized in the liver, that is, activated, inactivated, or transformed into metabolites with other partial effects. The chemical structures of these compounds and the combination with estrogens determine not only their binding affinity to the steroid receptor but also their binding to serum proteins, their distribution and storage in the organism, and their metabolism in the target tissues.[3, 4] Furthermore, both receptor concentration and receptor affinity play important roles in determin-

Table II. Effects of various synthetic progestogens*

Parameter	*Norethisterone*	*Levonorgestrel*	*Desogestrel*	*Norgestimate*	*Gestodene*
Ovulation inhibition (mg/day)	0.5	0.05-0.1	0.06	0.25	0.04
Transformation dosage (mg/cycle)	100-150	5-6	0.4-2.5	~5-10	2-3
Menstrual delay test (mg/day)	10-15	0.25-1.0	~0.25	5	~0.2
Binding affinity† to the progesterone receptor (%)	305	628	11 3-keto-desogestrel ~628	400-500	?
Maintenance of pregnancy	(+)	+	+	+	+
Estrogenic effects	+	0	0	0	0
Antiestrogenic effects	(+)	+	+	(+)	+
Androgenic effects	+	+	(+)	(+)	(+)
Antiandrogenic effects	0	0	0	0	0

*Some data were obtained from the pharmaceutical companies.
†Relative binding affinity to cytosol of human uterine tissue; progesterone = 100%.

Table III. Influence of new monophasic pills on carbohydrate metabolism

		Reactions to oral glucose tolerance test		
		Glucose tolerance test		
Progestogen	*Combination*	*Glucose*	*Insulin*	*References*
Levonorgestrel	30 μg EE 150 μg LNG	Normal to slight increase	Increase	Irsigler et al. (1982)[6] Skouby et al. (1983)[1]
Desogestrel	30 μg EE 150 μg DSG	Normal to slight increase	Increase	Schijf et al. (1983)[7]
Norgestimate	35 μg EE 250 μg NG	Normal to slight increase	Increase	Kaiser et al. (unpublished data)
Gestodene	30 μg EE 75 μg GTD	Normal to slight increase	Increase	Rabe and Runnebaum (unpublished data)

LNG = Levonorgestrel; EE = ethinyl estradiol; DSG = desogestrel; NG = norgestimate; GTD = gestodene.

Table IV. Influence of triphasic pills on carbohydrate metabolism

		Effect on oral glucose tolerance test		
		Glucose tolerance test		
Progestogen	*Combination*	*Glucose*	*Insulin*	*References*
Levonorgestrel	Triphasic	No significant effects on glucose and insulin		Irsigler et al. (1982)[6]
Norethisterone	Triphasic	No significant effects on glucose and insulin		Rabe et al. (1985)[8]

ing biologic effect. The progestogens can competitively or noncompetitively accelerate or inhibit the activity of many enzymes, and in this way, change the metabolic processes in the target organ, the liver, or another organ.[3, 4] Finally, the effects of the progestogens depend on the synergistic influence of the estrogens. The estrogens induce the synthesis of the progesterone receptors and thus create the basis for progestogen action.[5] Estrogens are responsible for the proliferation of the endometrium, whereas progestogens lead to a transformation.

Because of the large number of progestogen effects, which are partly created synergistically with estrogen, it is very difficult to characterize a particular progestogen (Table I). General evaluation is based on whether the substance has a progestogenic, estrogenic, antiestrogenic, androgenic, or antiandrogenic effect. The various metabolic effects of a particular progestogen in combination with estrogen are also important.

The new progestogens are strongly progestogenic with respect to ovulation inhibition, endometrium morphology, binding affinity to the progesterone receptor, and pregnancy maintenance (Table II). Levonorgestrel and the new third-generation progestogens, such as

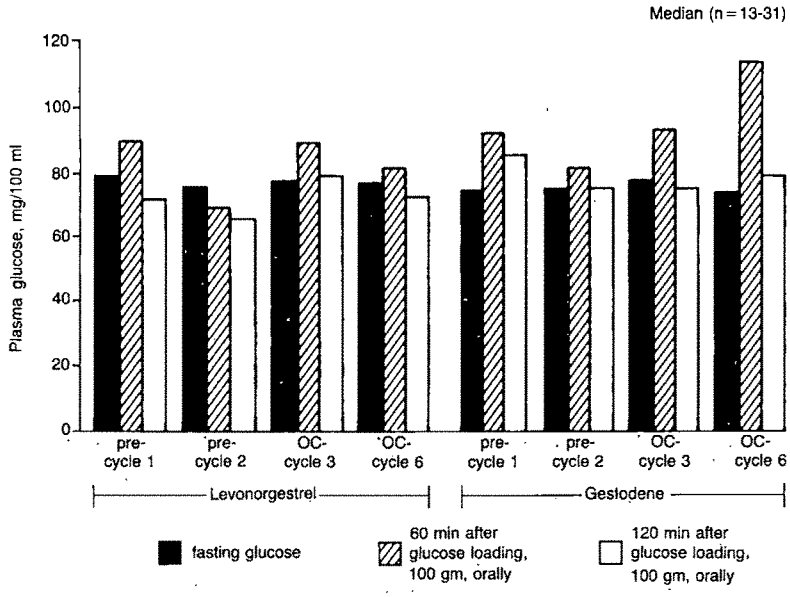


Fig. 1. Effects of levonorgestrel and gestodene on glucose response to a glucose load. (*OC* = Oral contraceptive.)

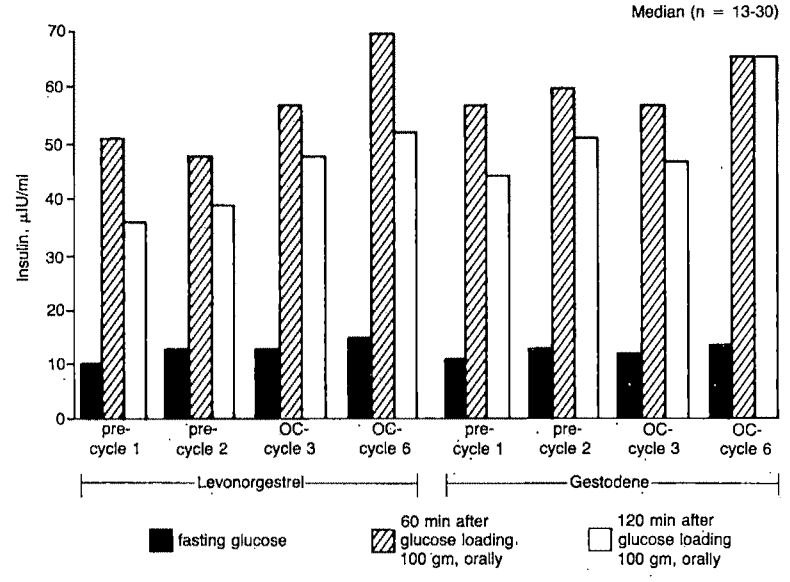


Fig. 2. Effects of levonorgestrel and gestodene on insulin response to a glucose load. (*OC* = Oral contraceptive.)

desogestrel, norgestimate, and gestodene, are real progestogens as judged by their pregnancy maintenance effects. They have no estrogenic effect, but, rather have pronounced antiestrogenic effects. Their androgenic effects are not clinically relevant, and they have no discernible antiandrogenic effects.

Carbohydrate metabolism

In comparing the influence of different oral contraceptives on carbohydrate metabolism (Table III), it is important to consider that factors independent of the pill, such as race, nutrition, and life-style, also play a role. Furthermore, the dosage, steroid content, and formulation of the particular pill may influence fasting glucose, glucose tolerance, kidney tolerance for glucose, and pancreatic insulin secretion. The effects of oral contraceptives on carbohydrate metabolism are assessed by measuring fasting glucose levels and performing an oral glucose tolerance test with measurements of glucose and insulin.[6-8] Pills containing the progestogens levonorgestrel and gestodene exert small effects on glucose and cause only minor increases in

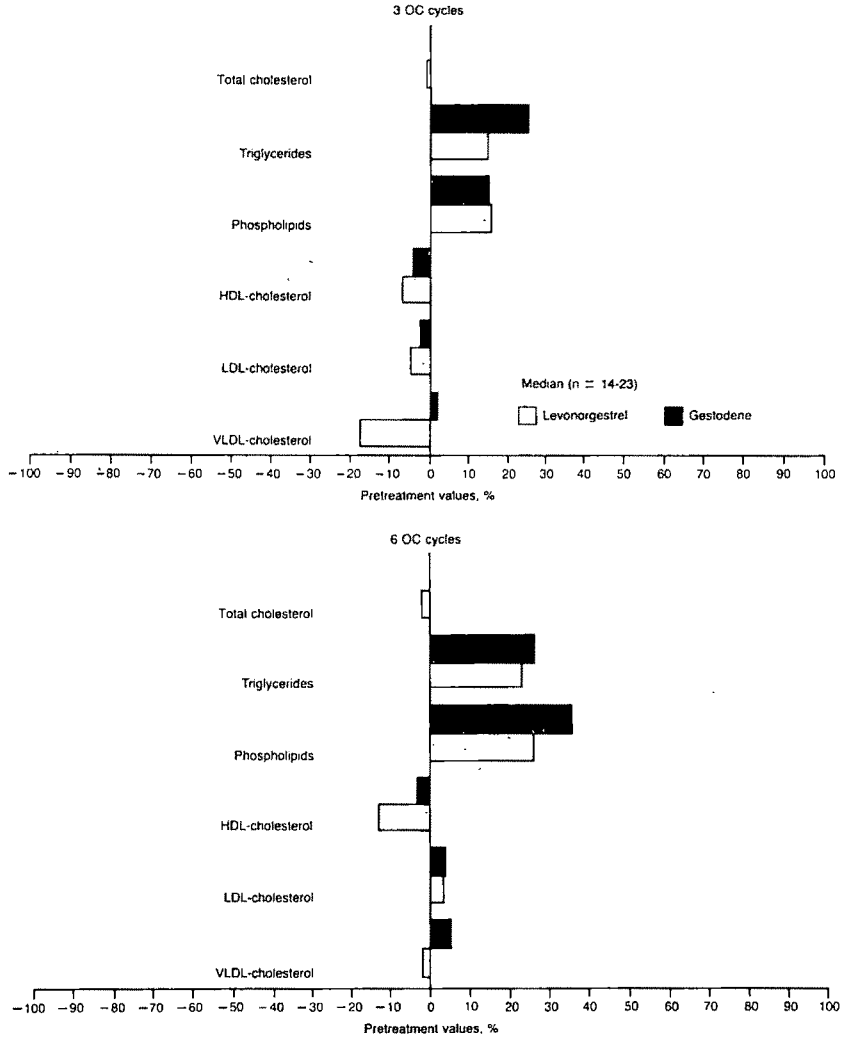


Fig. 3. Effects of levonorgestrel and gestodene on lipid metabolism. (*OC* = Oral contraceptive; HDL = high-density; LDL = low-density; VLDL = very low–density lipoprotein.)

Table V. Influence of monophasic pills on lipid metabolism

Progestogen	*Triglyceride*	*Total cholesterol*	*HDL-cholesterol*	*References*
Levonorgestrel (30 μg EE, 150 μg LNG)	Normal to 20% increase	Normal range	Normal range	Skouby et al. (1983)[1]
Desogestrel (30 μg EE, 150 μg DSG)	Normal to 20% increase	Normal range	Normal to slight increase	Mall et al. (1983)[9] Fotherby (1985)[2]
Norgestimate (30 μg EE, 250 μg NG)	20%-50% increase	Normal range	Normal to slight increase	Kaiser et al. and Anderson et al. (unpublished data)
Gestodene (30 μg EE, 75 μg GTD)	20%-30% increase	Normal range	Normal range	Rabe and Runnebaum (unpublished data)

EE = Ethinyl estradiol; LNG = levonorgestrel; DSG = desogestrel; NG = norgestimate; GTD = gestodene.

insulin level (Figs. 1 and 2). It is difficult to determine whether a minor deterioration in glucose tolerance that is associated with long-term pill use actually represents a danger to health, especially the health of the cardiovascular system (Table IV).

Lipid metabolism

Lipid metabolism is an important aspect in analyzing cardiovascular risk associated with oral contraceptive use. However, it must be kept in mind that risk factors such as smoking, hypertension, hyperlipidemia, adi-

posis, diabetes mellitus, and cardiovascular illness play a decisive role. Furthermore, various influences of oral contraceptives on lipid metabolism must be considered. Unfavorable effects, especially on the concentration of high-density lipoprotein cholesterol, may be genetic, fat dependent, or influenced by smoking.

The new progestogens in monophasic pills have only a low impact on total cholesterol and high-density lipoprotein cholesterol levels (Fig. 3).[1, 2, 9, 10] However, they exert a distinct effect on triglyceride concentrations (Table V), although the clinical importance of this finding is as yet uncertain.

Blood pressure

Although estrogen-progestogen mixtures may affect blood pressure regulation, long-term clinical trials show that the new progestogens, such as norgestimate and especially gestodene, do not change blood pressure. Animal experiments have proved that gestodene favorably influences the renin-angiotensin-aldosterone system, and it is possible that these effects also occur in humans.

Coagulation

The exact extent to which progestogen type and dosage are responsible for changes in the blood clotting system has not been fully elucidated. Low synergistic or antagonistic effects of progestogens have been observed, depending on the partial antiestrogenic properties of the particular compound. The low-dose oral contraceptives have only a low impact on blood clotting.[11, 12]

Directions for future research

The pharmacologic and clinical profiles of the new synthetic progestogens, gestodene and norgestimate, have aroused much interest. In low-dose monophasic combination pills, these substances have only little effect on carbohydrate metabolism and different effects on lipid metabolism. The pronounced effects on triglyceride and phospholipid concentration must still be studied. The changes in the blood clotting system seem to be limited.

Some 5% of the healthy women who took part in our pill studies exhibited marked changes in certain parameters of lipid metabolism and blood clotting. Future studies of oral contraceptives might focus more attention on those persons in whom lipid metabolism or the blood clotting system reacts strongly to the pill.

To date clinical experience indicates that the new progestogens are well accepted by patients and are associated with a low (5% to 6%) incidence of spotting.

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PART 2: ALTERNATIVE METHODS OF CONTRACEPTION

Introduction

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The development of alternative methods of contraception is reaching a watershed. Many of the theoretical innovations of the last decade are now being translated into practice in clinical trials.

The dynamism in today's contraception arena was evidenced by five progress reports presented during the second half of a Contraception Plenary Session at the XII World Congress of Fertility and Sterility in Singapore, October 28, 1986. One especially promising approach under investigation involves the use of antiprogesterones for fertility control. Still another cause for excitement is the fact that after a decade of intensive testing, four birth control vaccines are currently undergoing clinical trials. The male contraceptive gossypol also represents an interesting area, although further research will be necessary to determine whether associated toxicity can be circumvented at contraceptive doses. Natural family planning has evolved considerably, although it remains to be seen whether the use-effectiveness of this approach can be increased so as to match its high method-effectiveness. Yet another important advance, which promises to have worldwide application, is the long-acting subdermal levonorgestrel implant (Norplant).

The international developments discussed in these proceedings indicate that we have reason to be optimistic. As we approach the beginning of the next century, we should see a much broadened array of choices for couples desiring family planning assistance.

From the Karolinska Institute.

Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs

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Berlin, West Germany, and Beijing, China

Three antiprogestogens of the RU 38.486 type, RU 38.486, ZK 98.734, and ZK 98.299, were studied at different stages of pregnancy in the guinea pig. Treatment starting on postconception day 4 completely prevented nidation; all three compounds had comparable inhibitory potency. Treatment after nidation, starting on postconception day 8, induced decidual collapse and bleeding, but embryonic tissue was retained in nidation sites. In contrast to results in animals in nonfertile cycles, luteolysis was not induced, indicating that antiprogestogens lack the ability to induce uterine prostaglandin synthesis/liberation. On postconception day 43, RU 38.486 showed marginal abortifacient activity. The other compounds induced expulsion more rapidly and at a higher rate. The comparatively pronounced antiglucocorticoid activity of RU 38.486 may account for this difference. With RU 38.486, a high level of uterine prostaglandin sensitivity and a cervical ripening were induced consistently and fast; spontaneous labor, on the other hand, occurred after several days, if at all. Complete uterine evacuation was induced within hours by otherwise inactive doses of sulprostone in various combinations with ZK 98.299 and RU 38.486 but surprisingly not with ZK 98.734. A single dose of ZK 98.299 induced an approximately thirtyfold increase in uterine prostaglandin sensitivity within 24 hours, exceeding that present before term, but did not induce spontaneous labor. This is evidence that endogenous prostaglandins were not activated, analogous to perinidation stages. Observation of antiluteolytic activity of antiprogestogens in nonpregnant animals is considered of major theoretical importance in this context. It seems that inhibition of progesterone leads to suppressed uterine prostaglandin liberation. The same effect in pregnancy could explain the inability of the uterus to expel a seriously compromised conceptus. In conclusion, we suggest that progesterone is a stimulator rather than a depressor of uterine prostaglandins in the late luteal phase and pregnancy. The ability of the conceptus to neutralize this stimulatory action of progesterone is considered to be essential for the rescue of the corpus luteum and uterine motor quiescence in the guinea pig. The clinical significance of these findings is that the high frequency of incomplete abortions and protracted, sometimes heavy bleeding in pregnant women treated with RU 38.486 may reflect decidual compromise and simultaneous uterine prostaglandin deficiency, as found in our animal model after progesterone blockage.
(AM J OBSTET GYNECOL 1987;157:1065-74.)

Key words: Antiprogestogens, effect in pregnant guinea pigs, myometrium, endometrium, prostaglandin synthesis/liberation

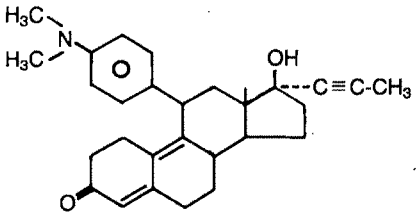
Csapo[1] theorized that the elimination of progesterone in pregnancy should automatically lead to pregnancy termination by explusion of the uterine contents. Since competitive progesterone antagonists became available as a result of the pioneering work performed by the scientists of Roussel-Uclaf,[2-4] it has become possible to reevaluate this concept critically in laboratory animals.

In spite of the confirmation of the abortifacient effects of RU 38.486 and other antiprogestogens in all evaluated animal species, certain phenomena require more detailed consideration. For example, in humans[5] these drugs often have produced incomplete abortions or have failed to induce expulsion as pregnancy advances.

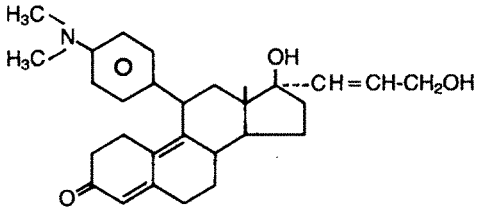
The effects of different antiprogestogens of the RU 38.486-type can be quite different. In light of this, we studied the effects of three steroids—RU 38.486 and two chemically related compounds (Fig. 1)—in guinea pigs at different stages of pregnancy. Compared with RU 38.486, the other two compounds have, among other alterations, side-chain modifications that consistently reduce their antiglucocorticoid activity.[6] A reduction in this activity may provide better tolerance.

In addition, there is some evidence of an interaction between the progesterone-antagonistic and antiglucocorticoid activity of these drugs at the uterine level. RU 38.486, as assessed by antithymolytic activity, has more antiglucocorticoid activity than the other two compounds. ZK 98.734 is the least active in this respect. It is important to note that oral administration of all three drugs leads to an *elevation* of antiglucocorticoid activity but no simultaneous increase in progesterone-antagonistic activity.

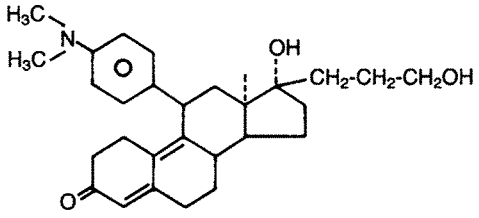
From the Research Laboratories of Schering Berlin/Bergkamen and the National Research Institute for Family Planning, Beijing.



RU-38.486
11β-(4-Dimethylaminophenyl)-17β-hydroxy-17α-(1-propinyl)-4,9(10)-estradien-3-one



ZK 98.734
11β-(4-Dimethylaminophenyl)-17β-hydroxy-17α-(3-hydroxy-prop-1(Z)-enyl)-4,9(10)-estradien-3-one



ZK 98.299
11β-(4-Dimethylaminophenyl)-17α-hydroxy-17β-(3-hydroxypropyl)-13α-methyl-4,9-gonadien-3-one

Fig. 1. Chemical structures of tested antiprogestogens.

Inhibition of nidation

Antiprogestogen treatment prevented nidation on postconception days 4 to 6, that is, before the day of nidation. A collapse of nidation sites was induced by treatment on days 8 to 11 of pregnancy. These effects were superior to those of castration, which does not terminate pregnancy at these early stages.[7] In guinea pigs, there seems to be embryonic progesterone secretion at the latest by the time of nidation, which permits nidation in the absence of luteal progesterone. The progesterone antagonists may be superior to castration due to their ability to neutralize this trophoblastic progesterone in addition to antagonizing luteal progesterone.

At both investigated perinidation stages, we found that maximum effects of all tested steroids were achieved at a dose of 10.0 mg/animal/day with subcutaneous administration. At a dose of 1.0 mg, there were no inhibitory effects. Incomplete and variable effects were seen at a dose of 3.0 mg; this finding suggests that 3.0 mg is the threshold dose for all three compounds.

This more or less comparable activity of the three steroids is in distinct contrast to their effects in later stages of pregnancy. Important additional information with regard to the mode of action was obtained from histologic investigations and the evaluation of serum progesterone. Histologic examination revealed that embryonic tissue was still present at degenerating nidation sites. This finding of incomplete abortion indicates that myometrial activation and mechanical evacuation did not take place. Furthermore, lack of effect of antiprogestogens on serum progesterone levels suggests that they did not induce uterine prostaglandin liberation, which would have exerted a luteolytic effect[8,9] (Fig. 2).

Myometrial effects of prostaglandins and antiprogestogens

The guinea pig model. This species was selected primarily because of some physiologic similarities to human pregnancy, for example, a luteoplacental shift of progesterone secretion and a high level of circulating progesterone until parturition. On postconception day 40, castration or luteolysis is possible without consequent abortion.

Moreover, we found an analogy between the uterine motor responses of the pregnant guinea pig to natural and artificial prostaglandins and those in human pregnancy.[10] Uterine motor activation leading to expulsion could also be achieved in the presence of high circulating progesterone levels. In this respect, the guinea pig was similar to monkeys but different from rats, in which the effects of the prostaglandin sulprostone on pregnancy were abolished by exogenous progesterone. The guinea pig model was very important for the discovery of sulprostone.[11]

The guinea pig uterus can be triggered to expel its contents at the earliest on day 20 of pregnancy. From this point, the oxytocin sensitivity increases progressively until it has risen by a factor of approximately 100

at parturition. The sensitivity for prostaglandin E_2 (PGE_2) and sulprostone increases mainly after day 40 by a factor of 10.

No such trend was seen for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The dose-response curves of $PGF_{2\alpha}$ were practically the same in the early, middle, and late stages of pregnancy. From this we conclude that PGE_2 and $PGF_{2\alpha}$ have different roles in the activation of the quiescent myometrium. We believe that $PGF_{2\alpha}$ is the "terminal" prostaglandin and that PGE_2 may act as a trigger for $PGF_{2\alpha}$.

Another aspect of pregnancy in the guinea pig that is analogous to human pregnancy is the development of practically absolute estrogen resistance around day 20. Around day 40, even 10 mg of estradiol was tolerated; only the rate of dead fetuses was raised.[12]

Antiprogestogens alone. The first steroid in our hands that was able to induce abortion by expulsion in advanced pregnancy in the guinea pig was RU 38.486. As can be seen in Fig. 3, several days elapse before expulsions occur. In this experimental situation, important differences emerged between the tested compounds. In the case of RU 38.486, we found that even very high doses, such as 30.0 mg/animal/day administered subcutaneously, failed to induce expulsions in more than 50% of the animals. On the other hand, ZK 98.734 showed dose-dependent effects and was faster acting than RU 38.486. All the animals aborted at the highest tested dose of 30.0 mg/animal/day administered subcutaneously.

The evaluation of oral activity confirmed these differences among the three compounds. We often observed a fall in the dose-response curve at high doses. At the highest tested dose of RU 38.486, only one out of six animals aborted. Most of those animals that did abort had prolonged abortions. Our studies with prostaglandin combinations (see below) indicate that low oral bioavailability of RU 38.486 probably does not account for this low activity.

Antiprogestogen/prostaglandin combinations. The low level of effectiveness of RU 38.486 and the long latency of expulsions compared with those after sulprostone prompted us to evaluate antiprogestogen/prostaglandin combinations. We studied both simultaneous and sequential treatments. Sulprostone was formulated in oil for simultaneous treatment (0.03 mg/day administered subcutaneously). For sequential treatment, a single 0.1 mg dose of sulprostone in saline solution was given 24 hours after the last antiprogestogen dose.

There were strong synergistic effects between some of the antiprogestogens and sulprostone. For example, a much higher number of animals aborted after simultaneous administration of RU 38.486 and marginal sulprostone doses than after administration of the progesterone antagonist alone. In addition, expulsion occurred much earlier after the combination (Fig. 4).

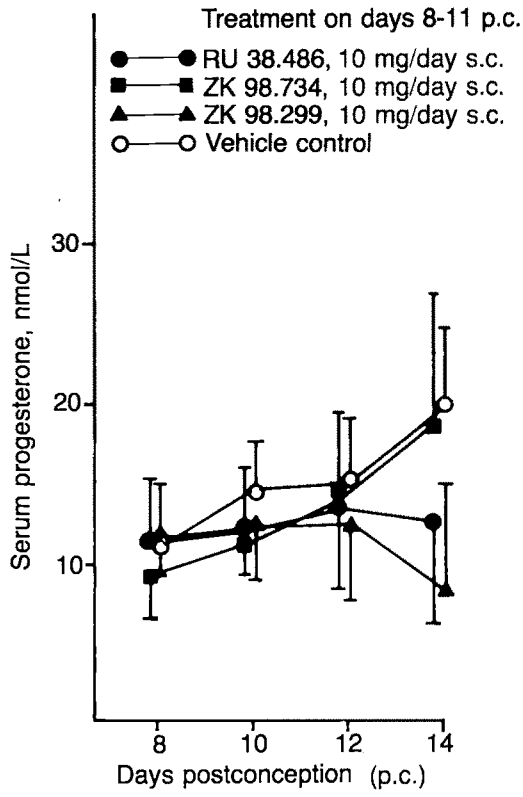


Fig. 2. Serum progesterone levels in aborting guinea pigs.

Surprisingly, this synergism did not apply to all substances to the same extent. In particular, ZK 98.734, which was fairly active when given alone at a dose of 3.0 mg/day administered subcutaneously, was not distinctly more active or faster acting when given in combination with sulprostone. On the other hand, even marginally active doses of RU 38.486 were synergistic.

Sulprostone treatment after antiprogestogen priming tended to lead to the rapid onset of expulsive behavior and the expulsion of conceptuses within a few hours. For example, supplementary sulprostone treatment after RU 38.486 and ZK 98.299 resulted in expulsion of conceptuses within 6 hours.

The different behavior of the above compounds was also confirmed in this sequential design. Again, ZK 98.734 induced expulsion at approximately the same time (most often, more than 48 hours after the last dose) with or without the prostaglandin.

The same pattern was seen after subcutaneous and oral application of the progesterone antagonists RU 38.486 and ZK 98.734. These data also show that RU 38.486, which is not very active when given alone orally, is fully active when combined with sulprostone (Fig. 5).

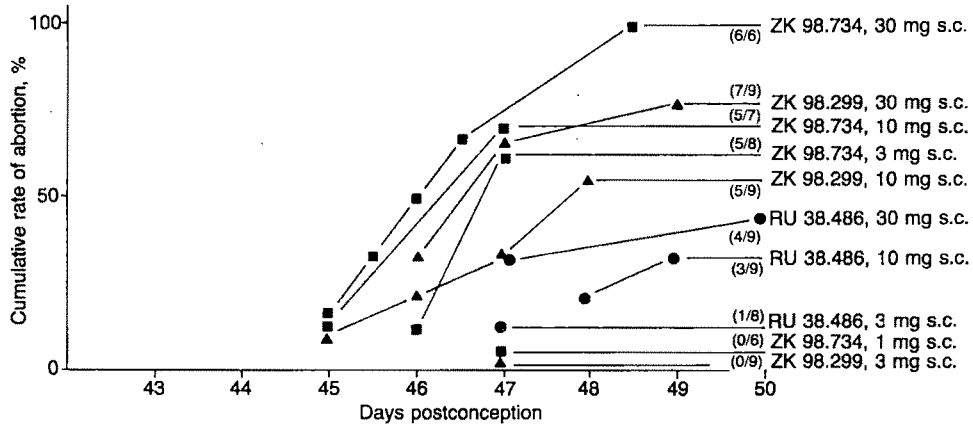


Fig. 3. Cumulative rate of abortions in guinea pigs after antiprogestogen treatment on postconception days 43 and 44.

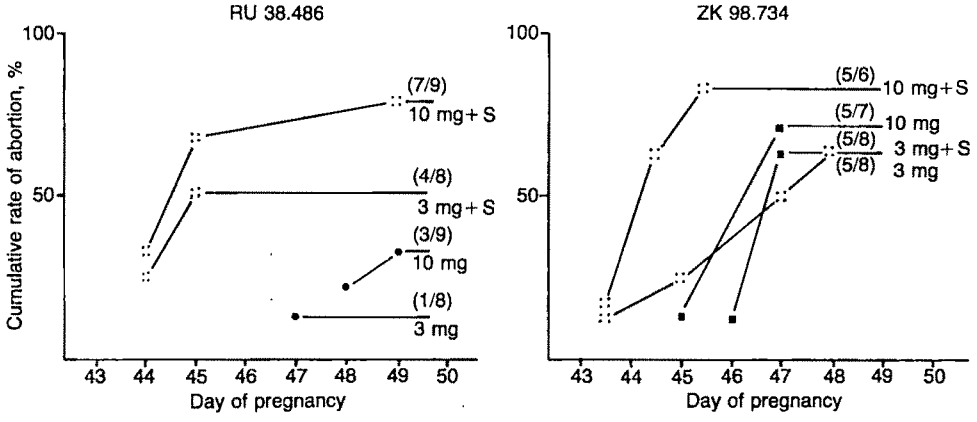


Fig. 4. Simultaneous antiprogestogen/sulprostone *(S)* (3 mg) treatment in guinea pigs; treatment was on postconception days 43 and 44.

Quantitative assessment of myometrial prostaglandin sensitivity. ZK 98.299 was found to be the most synergistic of the three compounds. Its activity was extraordinary in simultaneous and sequential combination regimens.

In order to establish the optimum duration of antiprogestogen priming, we compared the effects of 1 day's and 2 days' priming with a standard dose of 10.0 mg of ZK 98.299 per day administered subcutaneously and graded doses of sulprostone, injected 24 hours after the last antiprogestogen dose. There was no difference between the 1 day's and the 2 days' priming. This means that the uterus was completely sensitized within 24 hours. Above a well-defined threshold dose of sulprostone (that is, 0.01 mg administered subcutaneously), rapid expulsions and complete abortions occurred within 8 hours. This corresponds to an elevation of sulprostone sensitivity by a factor of 30 (Fig. 6). This uterine responsiveness is at least three times higher than that established in the immediate prepartum period (treatment on postconception days 63 and 64).

This dramatic myometrial sensitivity to the single ZK 98.299 injection was in sharp contrast to the absence of spontaneous abortion when subthreshold doses of prostaglandin were given. These data and those obtained with RU 38.486 demonstrate that some progesterone antagonists induce an "explosive" responsiveness of the myometrium long before the onset of "spontaneous" contractions, which may fail to occur at all.

This sequence of events is strong evidence that there is neither a preexisting "intrinsic uterine stimulant" nor sufficient activation of uterine prostaglandin to induce uterine contractions. Rather, our data suggest that uterine prostaglandins remain deeply suppressed for a couple of days. The failure to increase suppressed uterine prostaglandin secretion seems to us the key problem in understanding antiprogestogen actions in pregnancy.

Role of the glucocorticoid receptor in myometrial activation. In addition to the open question concerning antiprogestogen stimulation of uterine prostaglandins, the different behavior of antiprogestogens in the "myometrial" model used remains to be clarified. It seems un-

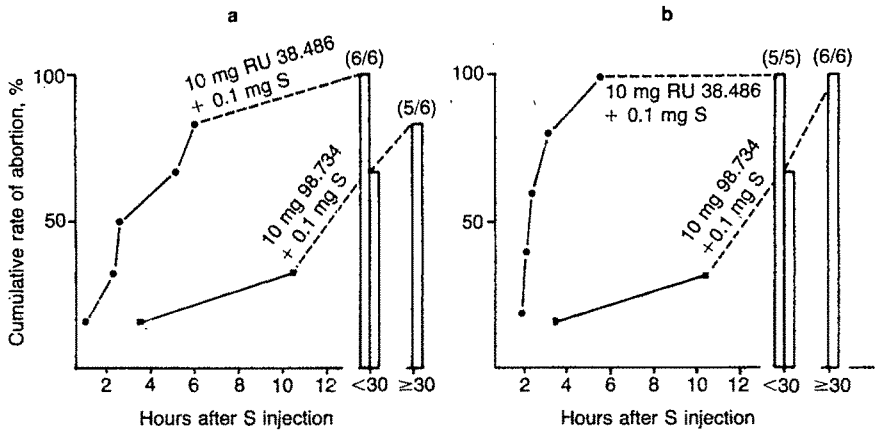


Fig. 5. Sequential antiprogestogen/sulprostone *(S)* treatment in guinea pigs: *(a)* subcutaneous application; *(b)* oral application. Antiprogestogen was given on postconception days 43 and 44; sulprostone, on postconception day 45.

likely that progesterone-antagonistic activity alone accounts for the different effects of the three compounds on myometrial prostaglandin sensitivity and the spontaneous onset of contractions.

We hypothesized that the interaction between the antiglucocorticoid activity and the progesterone-antagonistic activity of these drugs is favorable to myometrial sensitization and inhibitory to a later spontaneous onset of labor. This could explain the particular pattern of effects of RU 38.486.

In this context, we investigated the effects of dexamethasone in the guinea pig model in order to overcome the antiglucocorticoid activities of the antiprogestogens. Surprisingly, we found that dexamethasone inhibits the effects of sulprostone alone and reduces the activity of the antiprogestogens alone but increases the activity of the antiprogestogen/sulprostone combination. The onset of labor was advanced by approximately 24 hours. In the case of ZK 98.299, this combination brought about 100% abortions within 24 hours.

These data point to a potential role of the glucocorticoid receptor in myometrial activation. The nature of these effects is unknown, however, and the differences in the three compounds are not fully explained by their antiglucocorticoid activity.

Pilot studies with uterine pressure recordings. Intrauterine pressure was recorded in some animals during stimulation with oxytocic agents after antiprogestogen priming. This was done in conscious, freely moving animals after surgical intrauterine insertion of a microballoon, which was connected to a pressure tranducer by a fine polyethylene tube.

The individual data of these experiments must be regarded with some caution. Antiprogestogen-primed uteri were found to be extremely sensitive to mechanical irritation. Abortions were often recorded immediately after the operation. We found it necessary to insert the balloon before antiprogestogen treatment. Even this prompted expulsions before the expected time compared with the response in intact animals.

Nevertheless, we made some interesting observations

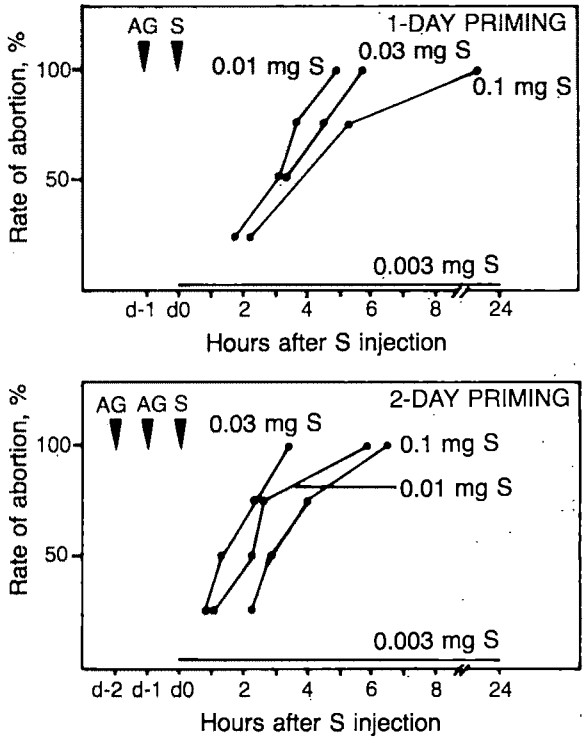


Fig. 6. Quantitative evaluation of uterine prostaglandin sensitivity in guinea pigs: priming with 10 mg of ZK 98.299 administered subcutaneously on postconception day 43 or days 43 and 44; single sulprostone injection administered subcutaneously 24 hours after last antiprogestogen *(AG)* injection.

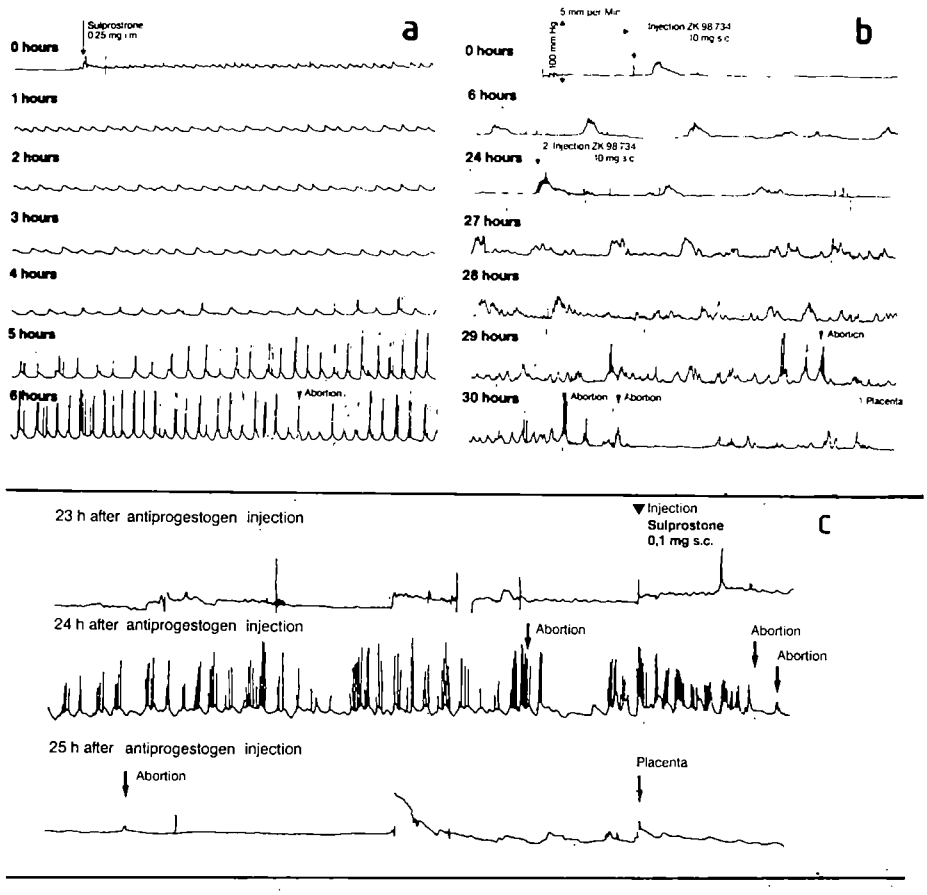


Fig. 7. Intrauterine pressure recordings in pregnant guinea pigs on postconception day 43 ± 1: *(a)* after a single sulprostone injection, *(b)* after two ZK 98.734 injections, and *(c)* with open silicone rubber catheter after priming with 10 mg of ZK 98.299 administered subcutaneously.

(Fig. 7). We found that a single injection of sulprostone induced very regular contractions after an initial elevation of resting intrauterine pressure. These led to an abortion within 6 hours.

Twenty-four hours after ZK 98.734 injection, there were irregular, very low-frequency contractions that lasted for several minutes and generated high intrauterine pressure. Expulsion occurred 30 hours after the first antiprogestogen treatment after a short phase of abdominal wall contractions (see spikes on Fig. 7, *b*) associated with uterine contractions.

Using a less traumatic device, namely, an open, fine silicone rubber tube, we recorded no spontaneous contractions 1 day after injection of 10.0 mg of ZK 98.299. A single 0.1 mg dose of sulprostone administered subcutaneously induced labor immediately and the expulsion of the first fetus within 1 hour.

This pattern is similar to the observations we made in intact animals; that is, the myometrium is highly responsive to stimuli but nevertheless quiescent. The very short duration of labor shows that the mechanical resistance of the genital tract is quite low. This will be explained by the effects of antiprogestogens at the level of the cervix (see below).

Satellite studies of $PGF_{2\alpha}$ in uterine vein blood and amniotic fluid. $PGF_{2\alpha}$ was estimated by radioimmunoassay in the plasma of uterine vein blood and in amniotic fluid at three times: before and after antiprogestogen treatment and at autopsy 6 hours after a final injection of a prostaglandin (PGE_2 or sulprostone) or at abortion. It was found that the repeated laparotomies had no effect on basal $PGF_{2\alpha}$ concentrations. There was also no consistent effect on basal $PGF_{2\alpha}$ levels in the two compartments after antiprogestogen treatment. A distinct elevation of $PGF_{2\alpha}$ was seen after the PGE_2 stimulation in vehicle-pretreated animals. This response was somewhat blunted in antiprogestogen-primed animals[13] (Fig. 8).

After ZK 98.734, the $PGF_{2\alpha}$ response seems to be particularly diminished. In spite of these comparatively low $PGF_{2\alpha}$ levels, however, some of the ZK 98.734–primed animals aborted. In contrast, the vehicle-

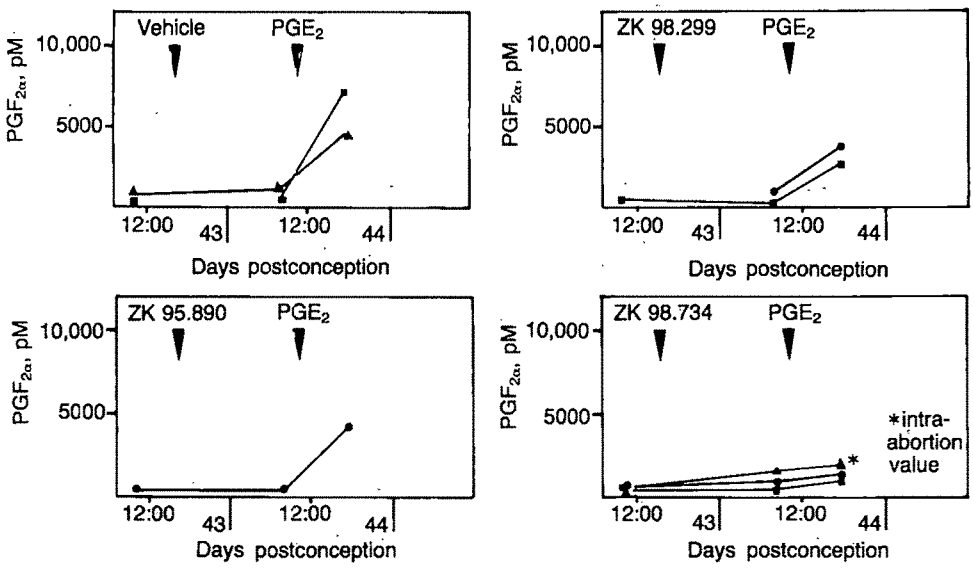


Fig. 8. Representative individual $PGF_{2\alpha}$ values in uterine vein plasma in pregnant guinea pigs (postconception days 43 and 44) after treatment with antiprogestogens (10.0 mg administered subcutaneously) and PGE_2 (1.0 mg administered subcutaneously).

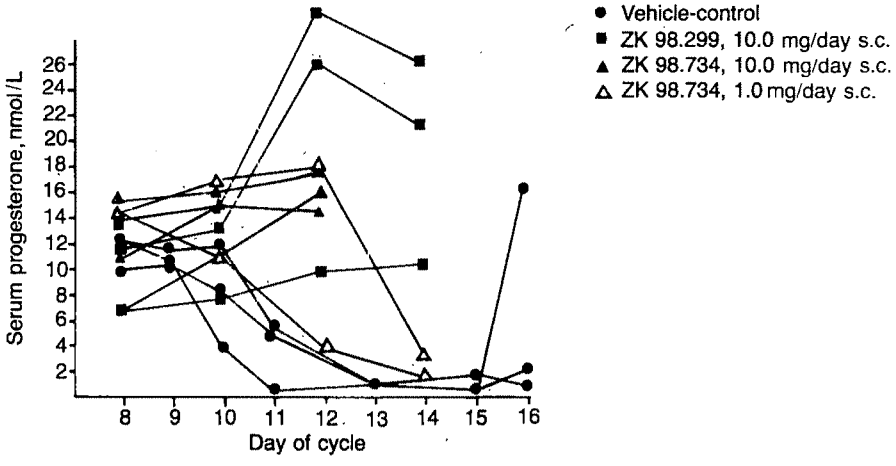


Fig. 9. Dose-dependent antiluteolytic effects of antiprogestogens in nonpregnant guinea pigs treated on days 8 to 11 of the cycle.

pretreated animals did not abort after exogenous prostaglandins until autopsy despite relatively high $PGF_{2\alpha}$ levels in uterine vein plasma.[13]

Antiluteolytic effects of antiprogestogens in nonpregnant guinea pigs. The described paradox, that is, maximum elevation of myometrial sensitivity associated with an absence of "intrinsic uterine stimulants," may have a surprising explanation. In the inhibition of nidation studies previously mentioned, some nonpregnant female animals were treated with antiprogestogens on days 8 to 11 of the cycle. The measurement of serum progesterone by radioimmunoassay revealed that in these ZK 98.299– and ZK 98.734–treated animals, the expected drop in progesterone levels at the end of the luteal phase did not occur (Fig. 9). Instead, these animals showed a pattern of serum progesterone that is typical of pregnancy. A study performed to check the validity of this observation confirmed the suspected antiluteolytic effects of ZK 98.299 in the guinea pig.[14] The dose-dependent antiluteolytic activity of ZK 98.734 and RU 38.486 has since been confirmed as well.

There is evidence in the literature that uterine prostaglandin, liberated by the nonpregnant uterus, destroys the corpora lutea in a nonfertile cycle. The inhibitory action of antiprogestogens on this event may

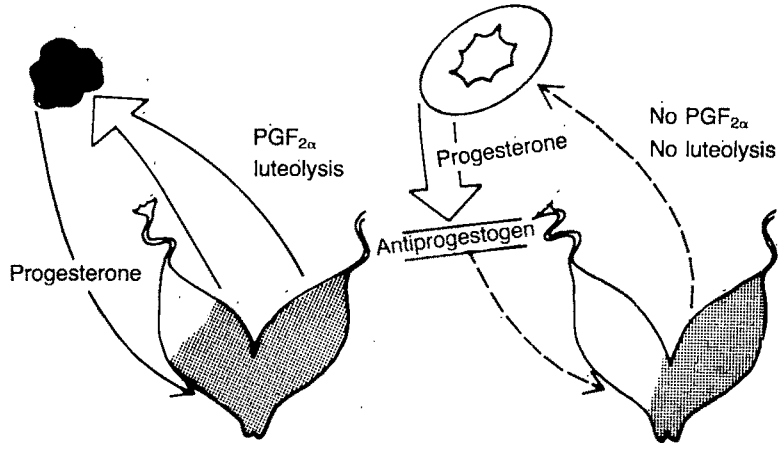


Fig. 10. Mechanism of antiluteolytic action of antiprogestogens: inhibition of progesterone-induced prostaglandin secretion.

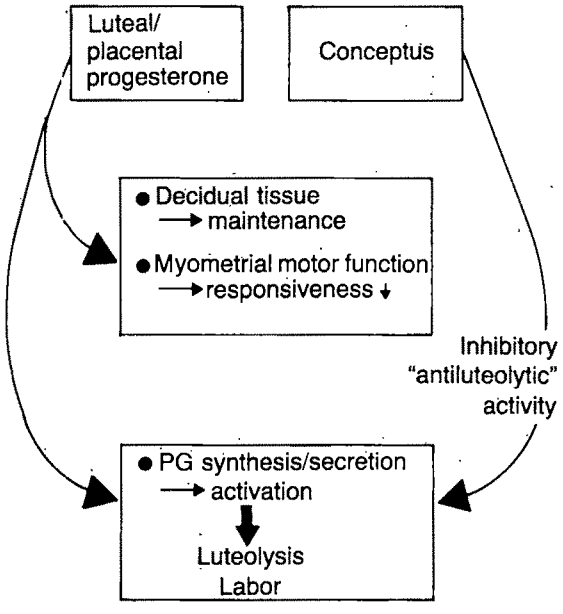


Fig. 11. Postulated paradoxical functions of progesterone in pregnancy.

therefore be explained as the inhibition of uterine prostaglandin liberation (Fig. 10).

If this action, rather than a direct or indirect luteotropic effect, does in fact occur, important questions arise about the role of progesterone in the control of uterine prostaglandins. As a hypothesis, we may assume that progesterone is a stimulator of uterine prostaglandins. The depression of uterine prostaglandins in pregnancy may be a function of the embryo, which first becomes manifest as its well-established antiluteolytic activity. Progesterone may represent a permanent stimulus of uterine prostaglandin generation in pregnancy that is under permanent embryonic suppression (Fig. 11).

Consequently, blockade of uterine progesterone in advanced pregnancy can be assumed to lead to suppressed prostaglandin generation; this is analogous to the events that take place during the luteal phase in a nonfertile cycle. This suppression of prostaglandins could lead to the inhibition of uterine motor activity as a result of the absence of an essential triggering mechanism, that is, uterine prostaglandins. A simultaneous collapse of decidual tissue, causing bleeding and myometrial sensitization, resulting from progesterone withdrawal at these target tissues would complete the observed "antiprogestogen syndrome."

Cervical effects of antiprogestogens in pregnant animals. There was a very rapid expulsion of conceptuses after sulprostone stimulation in antiprogestogen-primed animals. A ripening of the cervix, demonstrated by opening of the cervix and softening of cervical tissue, obviously plays an important role in this event. These changes were visible at vaginal inspection and were objectively measured in vitro.

We found spontaneous increases in the diameter of the cervix in the course of pregnancy, with marked preterm changes. There was a parallel softening of tissue, as assessed by reduced tension under stepwise extension. A dramatic dilatation and a softening of the cervix were recorded within 24 hours after a single dose of ZK 98.299 on day 43. Similar changes occurred even more rapidly around day 60 of pregnancy (Fig. 12).

Observation of the animals revealed other alterations in the birth canal, such as relaxation of the pelvic ligaments and opening of the symphysis pubis. These findings imply that relaxin may be a mediator of these effects.

We consider the observed effects of antiprogestogens at the cervix to be of potential clinical importance, in particular, for pregnancy termination at advanced stages or for the induction of parturition at term. It

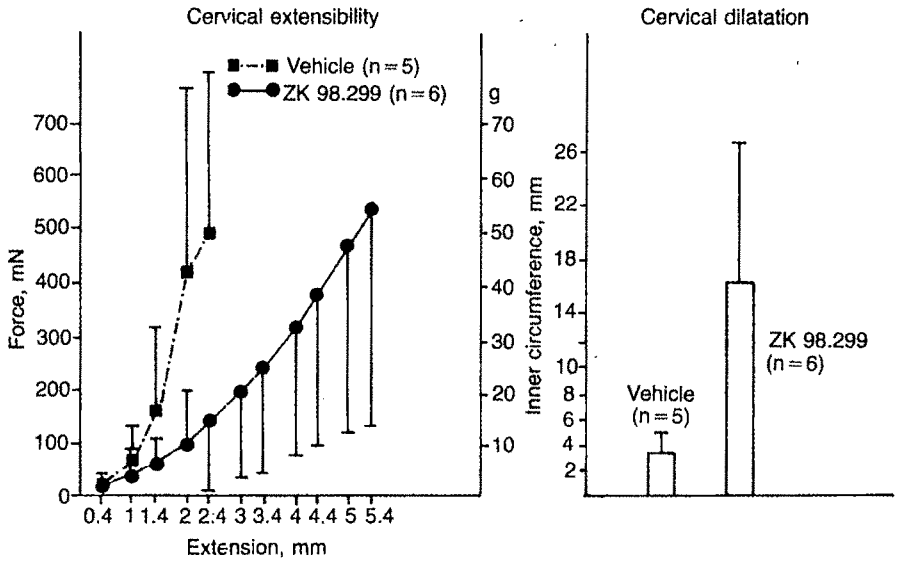


Fig. 12. Cervical extensibility and dilatation in pregnant guinea pigs after treatment with 0.3 mg of ZK 98.299 administered subcutaneously on postconception day 59, 5 to 6 PM; autopsy was performed on postconception day 60, 9 to 10 AM.

seems of particular importance that these effects can be obtained without simultaneous stimulation of uterine contractions.

Comment

Antiprogestogens were found to have important effects in various tissues in the pregnant uterus. Most of the observed effects on endometrial functions, such as prevention of nidation or collapse of nidation sites, are well explained by the known role of progesterone in nidation.

A less clear picture was obtained with regard to those functions that are related to myometrial motor activation, that is, the development of myometrial responsiveness to oxytocic stimuli on the one hand and the absence of endogenous stimuli that trigger the onset of labor on the other. Our studies revealed a rapid, dramatic elevation of myometrial responsiveness to exogenous prostaglandin and also to oxytocin, but there was no corresponding tendency of the uterus to go into labor spontaneously.[15]

This finding is in distinct contrast to the concept of Baulieu and Ulmann.[16] They postulated an increase in uterine prostaglandin as a consequence of the RU 38.486–induced blockade of the progesterone receptor and as a result of secondary effects, which lead to a decline in luteal progesterone production in humans, that is, a compromise of the conceptus and declining levels of chorionic gonadotropin.

On the basis of our data, we postulate exactly the opposite, the inhibition of uterine prostaglandin generation by progesterone-antagonistic activity. This seems to be reflected by a persistent suppression of uterine prostaglandin.

This notion is based on the absence of luteolysis in aborting animals in early pregnancy, the failure of highly sensitized uteri to go into labor, and the unchanged $PGF_{2\alpha}$ levels in uterine vein blood and amniotic fluid after antiprogestogen treatment. Inhibition of expected uterine $PGF_{2\alpha}$ liberation is evidenced by the clear antiluteolytic effect of antiprogestogens in the luteal phase in nonpregnant animals[14] and the blunted $PGF_{2\alpha}$ response after a PGE_2 or sulprostone stimulus in pregnant animals.[13]

The occurrence of "spontaneously" occurring expulsions after antiprogestogen treatment may not necessarily argue against the supposition of depressed uterine prostaglandin synthesis due to RU 38.486–type antiprogestogens. The abortions occurred quite late after treatment. At this time, there may be secondary changes, such as necrosis of decidual and trophoblastic tissue, and inflammatory responses that possibly coincide with stimulatory prostaglandin generation, independent of specific uterine control mechanisms.

It could also be of interest to investigate the pharmacokinetics of the tested compounds in relation to the established different time courses of expulsion.

If our assumptions—that uterine prostaglandin liberation is blocked by antiprogestogens and that this is due to their progesterone-antagonistic activity—are correct, it would be necessary to reconsider the role played by progesterone in the endocrine control of pregnancy. Its "classical" actions, namely, the lowering of myometrial responsiveness and the maintenance of

the nidation site, would be complemented by a paradoxical stimulatory activity on uterine prostaglandin liberation. This would imply that progesterone may have the potential to terminate a nonfertile cycle in the guinea pig or to stimulate uterine contractions unless embryonic inhibition overcomes this action (Fig. 11).

The cervical effects of antiprogestogens probably contribute greatly to the observed synergistic action of antiprogestogens and sulprostone by decreasing the mechanical resistance of the genital canal to the expulsive force of the uterus. The softening and opening of the cervix were also found to occur within a few hours. More pronounced and more rapid effects were found in preterm pregnancy than around day 40.

We infer from parallel observations of symphyseal changes that relaxin may be involved as a mediator of these responses. An elevation of relaxin levels may also be important in the depression of oxytocin secretion and the further inhibition of uterine contractility under antiprogestogens. Antiprogestogens appear to induce ripening of the cervix without stimulating myometrial contractions. This characteristic may be important in the potential use of antiprogestogens for therapeutic induction of abortion in advanced pregnancy or in obstetrics.

We suggest that the pattern of effects observed in clinical trials with RU 38.486 alone,[5] frequently ending in protracted bleeding and incomplete abortion, and in combination with sulprostone,[4] showing a strong antiprogestogen/prostaglandin synergism, is analogous to the effects we observed in midpregnancy in the guinea pig. This seems to be a good model for human pregnancy, particularly with regard to myometrial motor activation. Several potential approaches may be derived from the guinea pig model that could lead to the development of highly effective methods for medicinal pregnancy termination or for the induction of parturition.[17] This applies both to the selection of appropriate antiprogestogens to be used alone or appropriate antiprogestogen/prostaglandin combinations and to the design of treatment regimens that may arise from a better understanding of uterine physiologic and pharmacologic characteristics.

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Recent developments in immunocontraception

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The possibility of controlling fertility by antibodies inactivating key reproductive hormones has been amply demonstrated by active and passive immunization in primates. Four birth control vaccines directed against human chorionic gonadotropin are currently in early clinical trials. The nature of these vaccines and the underlying principles are described, as are the available results from clinical studies. The α- and β-subunits of human chorionic gonadotropin and the ovine gonadotropins have been cloned by recombinant deoxyribonucleic acid methods. A new breed of vaccines that combines the genes of gonadotropins linked to hepatitis B surface protein has been developed. The next generation of birth control vaccines is likely to be polyvalent and to have the ability to intercept fertility at more than one point. A number of monoclonal antibodies against human sperm have shown the presence of tissue-specific antigens and the possibility of preventing the fertilization of the egg. Inclusion of more than one carrier in the vaccine increases the percentage of high responders and accords immunoprophylactic benefits against more than one disease. Conjugates have also been developed to obtain high titers of antibodies against gonadotropin-releasing hormone with permissible adjuvants. This vaccine may have therapeutic applications in hormone-dependent cancers and precocious puberty. (AM J OBSTET GYNECOL 1987;157:1075-8.)

Key words: Immunocontraception, vaccines against human chorionic gonadotropin, vaccines combining genes of gonadotropins linked to hepatitis B surface protein, monoclonal antibodies against human sperm

Considerable research has demonstrated that fertility can be controlled by antibodies and/or cells involved in immune functions, and much progress has been made in the development of immunologic approaches to contraception.[1, 2] A large body of clinical data has shown that infertility is often attributable to antibodies against reproductive tract antigens.[3] In addition, a number of investigators have experimentally induced infertility by immunizing animals against reproductive tract hormones[4-8] and gamete antigens.[9, 10]

Particularly promising are vaccines that are directed against human chorionic gonadotropin (hCG), four of which are undergoing phase I clinical trials. This article focuses on recent developments in this area and also briefly reviews current prospects for the development of the next generation of birth control vaccines.

Counter hCG vaccines

hCG was chosen as a target antigen for several reasons. It is synthesized and secreted in appreciable amounts as a result of conception, and it is a reliable index of pregnancy, provided that production of hCG by trophoblastic cancers and hydatidiform moles can be excluded.

Recent evidence indicates that hCG is synthesized by embryos fertilized in vitro before implantation.[11] It is believed that hCG is important in establishing early pregnancy and, indeed, antibodies that abrogate hCG terminate pregnancy.[6, 12, 13]

Different strategies have been used to develop vaccines against hCG. Talwar et al.[14] chose the entire β-subunit of hCG as the antigen, whereas Stevens et al.[15] used a carboxyl-terminal peptide of β-hCG. Although carboxyl-terminal peptides do not produce antibodies that cross react with human luteinizing hormone (LH), they are poor antigens that require the use of strong adjuvants. Antibodies generated by carboxyl-terminal peptides have a low bioneutralization capacity as compared with antibodies that are produced by immunization with the entire β-hCG subunit.[16] In addition, carboxyl-terminal peptides are not recognized by the corpus luteum or Leydig cell receptors.[17] Limited in vivo studies have been carried out with carboxyl-terminal peptides of varying lengths, with some investigators reporting efficacy and others finding none.[18, 19] In contrast, the contraceptive efficacy of immunization with the whole β-hCG subunit has been confirmed in baboons and marmosets by a number of investigators.[6, 8, 13]

β-hCG–tetanus toxoid vaccine. The first of these vaccines to be developed consisted of β-hCG linked to tetanus toxoid. It has been tested in 63 women in six centers located in five countries.* The vaccine was given in four injections at 2-week intervals. It produced a

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Table I. Anti-hCG vaccines undergoing phase I clinical trials

*Vaccine**	*Adjuvant*	*Centers*	*No. of subjects*
β-hCG–TT	Alum; SPLPS* in first injection only	5 Centers in India 2 Centers in Finland and Sweden	30 18 + 15 Vehicle control
α-oLH–β-hCG–TT–CHB	Same as above	5 Centers in India	30
β-oLH–TT + β-hCG–TT–CHB	Same as above	5 Centers in India	30
37 Amino acid CTP β-hCG–DT	Arlacel A, squalene	1 Center in Australia	20 + 10 Vehicle control

α-oLH and β-oLH; α- and β-subunits of ovine LH; TT, tetanus toxoid; CHB, cholera toxin chain B; CTP β-hCG, C-terminal peptide of β-hCG; DT, diphtheria toxoid.

*SPLPS, Sodium phthalyl lipopolysaccharide.

reversible antibody response against both hCG and tetanus,[14] which in good responders lasted for 300 to 500 days.[20,21] Immunization did not affect ovarian function; ovulation was maintained with progesterone produced normally in the luteal phase. No significant side effects were noted.[22-24] The main limitation of this vaccine was the large intersubject variability in antibody titers; subjects who had low titers of hCG antibodies were not protected against pregnancy.

Improved vaccine formulations. Further research has led to the development of two new vaccine formulations. One of them consists of β-hCG annealed to a heterospecies (ovine) α-subunit of LH and linked to two carriers, namely, tetanus toxoid and cholera toxin chain B. The rationale for this approach is as follows: Gonadotropic hormones contain two subunits. Maximal bioactivity of the hormone is achieved when the two subunits are associated, as this conformation is read optimally by the target tissue receptor. Dissociation of the subunits leads to a drastic conformational change and loss of bioactivity. α-hCG is not usable, because it would cross react with human thyroid-stimulating hormone (TSH) and human follicle-stimulating hormone (FSH). β-hCG, however, anneals readily to α-subunits of gonadotropins of other species, and the resulting conformation has a steroidogenic potential superior to that of hCG.[25] Ovine α-LH does not produce antibodies that cross react with human TSH or FSH. The annealed antigen, however, has a significantly higher immunogenicity in bonnet monkeys than does β-hCG–tetanus toxoid.[26]

The carrier in these specially structured vaccines contributes to immunogenicity and to immunoprophylactic benefit. Tetanus toxoid is a good carrier, but response to tetanus toxoid varies from recipient to recipient. The purpose of using two carriers, rather than one, is to evoke a response in those persons who are hyporesponders to tetanus toxoid. The gonadotropin subunits linked to cholera toxin chain B evoked a higher response in bonnet monkeys that had low titers after immunization with tetanus toxoid–based vaccine.[27] For this reason, these two carriers were chosen for use in the formulations that are now undergoing clinical trial.

The second improved vaccine formulation is a mixture of ovine β-LH and β-hCG, each linked to a carrier–tetanus toxoid and cholera toxin chain B. The mixture is eight to 13 times more immunogenic than β-hCG–tetanus toxoid in monkeys and rodents.[28] An additional benefit of including ovine β-LH is to introduce antibodies that cross react with human LH. Partial neutralization of the LH surge does not impair ovulation, but in monkeys it leads to a reduced rate of progesterone production in the corpus luteum and a shortening of the luteal phase by about 2 days.[29] Clinical cases of infertility have been recognized in women who have a slightly shortened luteal phase and deficient corpus luteum. Thus the mixed vaccine represents an attempt to simulate this condition by evoking antibodies that intercept fertility at two points in the reproductive chain of events.

The Population Council, New York, has carried out extensive safety studies involving 60 rhesus monkeys that were hyperimmunized with ovine β-LH in Freund's complete adjuvant. Careful toxicologic studies and histopathologic examinations over a 5- to 7-year period of observation did not show any pathologic conditions of the pituitary gland or the kidneys or other untoward side effects.[30]

Clinical trials. Four vaccines are currently undergoing phase I clinical trials (Table I), and the relative merits and limitations of each should become evident within the next 6 to 9 months.

The overall objective of the trials in India is to select the most immunogenic formulation of the vaccine. Study goals include determining whether 100 or 500 μg of gonadotropin(s) is the preferable dose for immunization; investigating the characteristics of the antibodies, including their bioneutralizing ability; observing the effects of the vaccines on reproductive and other body functions; and assessing the duration and reversibility of the antibody response.

The vaccines were released in May 1986 after due

clearance by the Drug Controller of India and Toxicology Review Panels of the Indian Council of Medical Research. Centers that conducted the trials also obtained clearances from their respective ethics committees. As of September 15, 1986, 76 subjects were enrolled in the study. Before immunization, baseline parameters were established for 2 months in each patient. By October, 21 women had received one injection of the vaccine, nine had received two, and one had completed the course of three injections at monthly intervals.

In all women, antibodies against hCG were measurable in the circulation. The subject who had received three injections had a titer of 78 ng of hCG binding capacity per milliliter. The affinity of the antibodies for hCG was 3.4 $nmol^{-1}$. The antibodies did not cross react with human FSH or TSH but cross reacted (13%) with human LH.

Thus far, no significant side effects have been reported. One subject who showed sensitivity to tetanus toxoid was excluded from the study. Three of the 21 subjects experienced overnight pyrexia after immunization, but this complaint was not related to the dose. Trials of a three-dose regimen of our first vaccine, the β-hCG–tetanus toxoid vaccine, are being resumed by the International Committee on Contraception Research of the Population Council in two Scandinavian centers.

Other fertility control vaccines at advanced stages of experimental development

Counter gonadotropin-releasing hormone (GnRH). The decapeptide hormone GnRH is common to both sexes, and immunization against it likewise controls fertility in both sexes. Although the efficacy of immunization against GnRH with Freund's complete adjuvant was shown more than a decade ago,[31] it has since become possible to create a vaccine that induces antibody titers high enough to be bioeffective in male[32] and female[33] animals with the use of appropriate adjuvants.

The GnRH vaccine may also have medical applications in precocious puberty and in such hormone-dependent cancers as carcinoma of the prostate and some breast neoplasms. However, because the vaccine reduces the levels of testosterone in immunized animals, its use as a male contraceptive is unlikely. Nevertheless, androgens are currently being tested that can meet the additional testicular demand in male animals that are rendered infertile by immunization against GnRH.

Counter FSH. Active and passive immunizations against FSH result in reduced sperm counts in monkeys.[4, 34] In rhesus monkeys, spermatogenesis was restored after 4½ years of active immunization, despite high circulating antibody levels.[34] Bonnet monkeys, however, remained infertile after active immunization against FSH.[4]

The next generation of birth control vaccines

The possibility of obtaining proteins by recombinant deoxyribonucleic acid methods has sparked active interest in sperm antigens. For example, active immunization against lactic dehydrogenase C_4, a tissue-specific sperm protein, significantly reduces fertility.[35] Many monoclonal antibodies have been developed against human sperm antigens; some of these antibodies demonstrate the presence of sperm-specific epitopes that may have important applications for fertility control and are likely to be developed as birth control vaccines.

The use of more than one antigen in vaccines will offer the advantage of intercepting fertility at more than one point. Polyvalent vaccines that consist of more than one reproductive tract antigen linked to more than one carrier are being developed and tested in our laboratory as well as in other laboratories. Also notable is the development of constructs of birth control vaccines at the deoxyribonucleic acid level. The β-hCG gene has been inserted along with the gene coding for hepatitis B surface protein.[36] Vaccinia has also been proposed as a possible vector for the next generation of live recombinant vaccines.[37]

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Experiences with gossypol as a male pill

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In 1981 to 1983 we performed a clinical study with gossypol involving 152 participants, and in 1983 to 1985 we conducted another study of 120 participants. The first study was aimed at confirming gossypol's antifertility efficacy and determining the existence of side effects. The objective of the latter study was to find out whether the addition of a potassium salt supplement or a potassium-sparing agent could alleviate the side effect of hypokalemia. In both studies, the participants took a gossypol pill, 20 mg/day for 60 to 75 days for loading, and 50 mg/wk for maintenance. All participants were followed up for a year. The antifertility efficacy was found to be more than 90%, and the chief side effect was lowered serum potassium. In our 1983 to 1985 study, we concluded that since neither potassium supplementation nor triamterene solved the problem, it is very likely that gossypol is a nephrotoxic agent. With 1 year of gossypol treatment, serum testosterone and serum luteinizing hormone showed no change, whereas serum follicle-stimulating hormone showed some elevation after 6 months. The Shanghai researchers found that in their gossypol users, plasma and urinary β_2-microglobulin levels were elevated to a certain extent. However, 25 subjects in our 1983 to 1985 study showed no appreciable change. Our volunteers had stopped taking gossypol for more than 1 year. In 1986 we started a third study, which was aimed at finding the lowest antifertility dose to minimize possible renal toxicity. (Am J Obstet Gynecol 1987;157:1079-82.)

Key words: Contraception, male, gossypol, irreversible aspermia, hypokalemia, nephrotoxicity

In the 1970s, a multicentered clinical study involving nearly 10,000 men showed that gossypol was an effective male antifertility agent. Its adverse effects were hypokalemia[1] and irreversible aspermia in a few cases. However, this study was not entirely satisfactory, chiefly because of a lack of uniformity in research protocols and inaccuracies in the basic data.

Enthusiasm for clinical gossypol studies gradually declined in the 1980s. The open door policy in China and practical attitude toward scientific research have brought about more careful study design and stricter verifications of data. The multicentered collaborative study of gossypol has given way to single-centered studies of this agent.

Recently, at the Peking Union Medical College Hospital we have performed two clinical gossypol studies, the purposes of which were to investigate the phenomenon of hypokalemia. Our 1981 to 1983 randomized controlled study involving 152 men attempted to clarify whether gossypol actually causes hypokalemia and if so how frequently.[2] The objective of our 1983 to 1985 study of 120 men was to determine whether hypokalemia is a direct effect of gossypol or a secondary manifestation of renal toxicity. In a third study, we investigated the lowest antifertility dose in the hope of minimizing toxicity.

From the Peking Union Medical College Hospital, and The Rockefeller Foundation.

Incidence of gossypol-induced hypokalemia

In the 1981 to 1983 study, the mean serum potassium level gradually declined during 1 year of gossypol treatment (Table I). The decline was obvious almost immediately after the initial loading phase. Later, serum potassium level continued to drop until leveling off toward the end of 1 year.

Clinical hypokalemia, however, occurred only occasionally. Only one of the 152 patients who participated in our 1981 to 1983 study experienced weakness of the neck muscle. This symptom developed in the third month of the maintenance phase when the patient's serum potassium level had fallen to 2.6 mEq/L. The patient's electrocardiographic changes were also typical. Five other patients in this study also had serum potassium levels below 3.0 mEq/L but did not develop any clinical symptom. After cessation of gossypol treatment, the decline in potassium levels did not persist. When gossypol was withdrawn after 1 year of gossypol treatment, serum potassium levels rose to a certain extent but did not return to the initial level.

One-year follow-up study of semen analysis showed a 49% incidence of irreversible aspermia.

Mechanism of gossypol-induced hypokalemia

In our second study, we investigated whether hypokalemia can be prevented by the addition of a potassium salt supplement or a potassium-sparing diuretic. Our goal was to test the hypothesis of those

Table I. Serum potassium (mEq/L) in 152 gossypol volunteers*

Length of use	*Total*	*Mean* ($\bar{x}$)	*SE* ($\bar{x}$)	*95% Confidence interval*
Admission	152	4.29	0.03	4.23-4.35
End of loading	124	4.06	0.04	3.99-4.14
3 mo	118	3.93	0.04	3.85-4.01
6 mo	108	3.82	0.04	3.74-3.90
9 mo	95	3.86	0.04	3.78-3.94
12 mo	86	3.75	0.04	3.67-3.83

*F = 30.0305 ($p < 0.05$).

Table II. Serum potassium (mEq/L) in 120 gossypol volunteers

Follow-up	*Group 1*	*Group 2*	*Group 3*	*Group 4*
1	4.30 (30)	4.32 (30)	4.47 (28)	4.23 (30)
2	4.01 (21)	4.12 (20)	4.26 (26)	4.52 (30)
3	3.64 (18)	3.78 (18)	3.76 (25)	4.33 (30)
4	4.04 (16)	3.54 (16)	3.56 (23)	3.90 (29)
5	3.95 (16)	3.76 (16)	3.74 (24)	3.54 (28)
6	3.84 (14)	4.00 (13)	3.84 (24)	3.94 (25)
7	4.02 (14)	4.02 (14)	3.72 (22)	3.96 (27)

scientists who consider hypokalemia to represent a direct effect of gossypol on serum potassium, which could be averted by the addition of potassium.[1]

Our 120 participants were divided into four groups. The first group took gossypol only, the second group took gossypol plus potassium chloride, 0.5 gm three times a day, the third group took gossypol plus triamterene, 50 mg/day, and the control group took nothing. In the first, second, and third groups, serum potassium declined to similar levels, although these decreases were less marked than those in our 1981 to 1983 study (Table II). This difference might be explained by the fact that the loading phase of our 1983 to 1985 study was 60 days, whereas that of our 1981 to 1983 study was 75 days.

One of the 120 patients developed clinical hypokalemia with lower limb paralysis 1 month after completion of 1 year of treatment. This patient's serum potassium level fell to 2.0 mEq/L and his electrocardiographic findings were typical. Although the patient responded very well to potassium supplementation, his serum potassium level 1½ years after cessation of gossypol treatment was still as low as 3.6 mEq/L compared with a pretreatment serum potassium level of 4.2 mEq/L.

On the basis of these results, we concluded that gossypol-induced hypokalemia should not be taken lightly as a direct effect of electrolyte imbalance. Rather, it should be viewed as caused by changes in an electrolyte-regulating organ, namely, the kidney.

Because to the best of our knowledge most kidney function tests were either too inconvenient or insensitive, we did not perform any such tests in either the 1981 to 1983 or the 1983 to 1985 study. However, in early 1986, we learned that the β_2-microglobulin test was convenient and sensitive and that our Shanghai colleagues had found abnormal test results in most of their volunteers.[3] We therefore called back some of the participants in our two studies. Our preliminary results of β_2-microglobulin testing in 25 patients who participated in the 1983 to 1985 study showed that renal function was not greatly impaired. The test was performed 1 year after the cessation of 1 year of gossypol treatment.

Prevention of gossypol-induced hypokalemia

Based on these considerations, we began a third study in 1986, with the goal of finding the lowest antifertility dose to minimize possible renal toxicity. Our low-dose study involved 39 participants. The first group of six participants took 15 mg/day for 60 days for loading and 45 mg weekly for maintenance. The second group of 13 participants took 10 mg/day for 60 days for loading and 30 mg weekly for maintenance. In addition, we had two control groups consisting of 10 participants each.

At the end of 60 days, the antifertility efficacy (sperm count <4 million/ml) was 67% in patients who received 15 mg loading doses but was only 23% in the 10 mg group (Table III). If antifertility efficacy is measured by the hamster egg penetration test, failure of penetration at the end of 60 days was 100% with the 15 mg regimen and 80% with the 10 mg regimen (Table III). No pregnancy occurred in the 15 mg group after 4 months without protection; one pregnancy took place in the 10 mg group after 1 month without protection.

Serum potassium level in the 15 mg group showed a tendency to drop after 60 days and decreased further after 120 days (Table IV). However, the difference was not statistically significant when compared with the control group.

Blood and urinary β_2-microglobulin levels in both the 15 mg and 10 mg groups showed no significant changes (Table V).

Table III. Fertility status after 60 days in the low-dose gossypol trial

Group	*No. of cases*	*Sperm count <4 million*	*%*	*No. of cases*	*Hamster egg penetration <10%*	*%*
15 mg	6	4	67	5	5	100
10 mg	13	3	23	10	8	8

Table IV. Serum potassium (mEq/L) in the low-dose gossypol trial

Group	*No. of cases*	*Admission*	*60 days*	*p*	*120 days*	*p*
15 mg	6	3.92 ± 0.26	3.73 ± 0.27	>0.05	3.45 ± 0.12	<0.01
Control	10	3.86 ± 0.26	3.78 ± 0.24	>0.05	3.52 ± 0.12	<0.01
p			>0.05		>0.05	
10 mg	13	3.82 ± 0.27	3.62 ± 0.28	>0.05		
Control	10	3.78 ± 0.24	3.52 ± 0.12	>0.05		
p			>0.05			

Table V. β_2-Microglobulin in the low-dose gossypol trial

Group	*No. of cases*	*Serum β_2-microglobulin*		*Urine β_2-microglobulin*		*p*
		Admission	*60 days*	*Admission*	*60 days*	
15 mg	6	3.03 ± 0.06	3.06 ± 0.05	2.04 ± 0.21	1.98 ± 0.30	>0.05
10 mg	13	3.08 ± 0.09	3.09 ± 0.06	1.94 ± 0.28	1.91 ± 0.25	>0.05
Control	10	3.09 ± 0.21	3.07 ± 0.19	2.27 ± 0.10	2.24 ± 0.16	>0.05
p			>0.05		>0.05	

Based on these data, the 15 mg regimen appears to be the appropriate way of providing dependable antifertility action while at the same time avoiding possible damage to the kidneys. With the 10 mg regimen, the loading and maintenance doses seem too low to ensure antifertility action. Since the sample size of our low-dose study is small, further study with more participants is warranted.

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Natural family planning

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It is now well accepted that a woman can conceive from an act of intercourse for a maximum of only about 7 days of her menstrual cycle. The reliability of natural family planning depends on identifying this window of fertility without ambiguity. Several symptomatic markers, cervical mucus and basal body temperature, have been used extensively and with considerable success in most women but failures occur. Ovarian and pituitary hormone production show characteristic patterns during the cycle. Urinary estrogen and pregnanediol measurements yield reliable information concerning the beginning, peak, and end of the fertile period, provided that the assays are accurate and performed on timed specimens of urine. We have developed such enzyme immunoassays for urinary estrogen and pregnanediol glucuronides that can be performed at home. In the early versions of the assays, enzyme reaction rates were measured by eye, but more recently, a simple photoelectronic rate meter has been used. The final problem to be solved is not technologic but whether women are sufficiently motivated to expend the same time and effort each day for 10 days a month, with less cost, on fertility awareness as they spend on making a cup of tea. (AM J OBSTET GYNECOL 1987;157:1082-9.)

Key words: Natural family planning, cervical mucus, basal body temperature, urinary estrogen and pregnanediol assays, enzyme immunoassays for urinary estrogen, pregnanediol glucuronides

Natural family planning is defined as voluntary avoidance of intercourse by a couple during the fertile phase of the menstrual cycle to avoid pregnancy.[1] Natural family planning thus involves a continual awareness of fertility status, including the day of ovulation. Therefore many regard the use of this awareness to expedite pregnancy as being part of natural family planning.

The earliest form of natural family planning was the rhythm or calendar method. The day of ovulation was considered to occur 14 ± 2 days before the next menstruation, and the period of possible fertility was calculated from this by allowing for the fertilizing life of the sperm and for normal variations in cycle length.[2] The rhythm method served a generation well; although many unplanned pregnancies occurred, the alternatives available at that time also had high failure rates.

The situation changed dramatically with the introduction of hormonal contraception after the pioneering work of Pincus and Rock and several pharmaceutical firms. The virtual 100% effectiveness of the hormonal pill raised the population's expectations for family planning and also played a large part in the emancipation of women, enabling them to become as reliable wage earners as men.

In comparison, the rhythm method seemed too unreliable. At that time, many scientists doubted whether periods of infertility actually occurred during the human cycle. Indeed, many believed that ovulation could occur more than once and could be induced by copulation. The enormous increase in our knowledge of human reproduction since then has shown that these objections are incorrect and that the concept behind natural family planning is theoretically sound, namely, that there are definite periods of fertility and infertility during the menstrual cycle. Fig. 1 shows that the chances of conception from an act of intercourse are very low 6 days before ovulation, increase to a maximum during the day before ovulation, and fall to zero within 24 hours after ovulation.[3]

The scientific challenge then is to devise methods for identifying these periods of fertility and infertility with absolute certainty so that couples who use natural family planning have the same reliability as those using other modern methods of family planning. The main difficulty in natural family planning for motivated couples is not the periodic abstinence required, which may in fact enrich the union. Rather, it is the uncertainty in defining the fertile phase accurately, the excessive abstinence that is therefore required for maintaining adequate safety margins, and the loss of confidence resulting from unexplained pregnancies.

Symptoms

Fig. 2 shows the various indexes that have been applied to natural family planning. The rhythm calcula-

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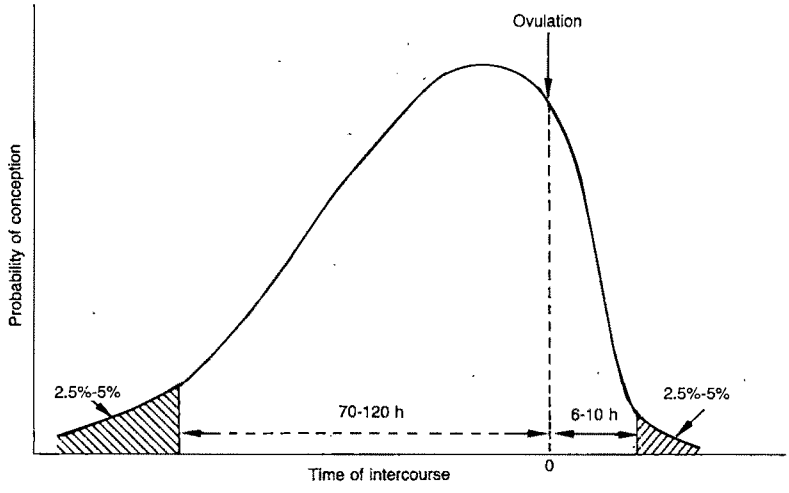


Fig. 1. Probability of conception *v*, the time of intercourse relative to ovulation in the human. (Reproduced with permission from Austin CR. Sperm fertility, viability and persistence in the female tract. J Reprod Fertil [Suppl] 1975;22:75-89.)

tions provide the background information on when ovulation can be expected in regular cycles.

Basal body temperature. The basal body temperature shows a characteristic rise of approximately 0.3° C after ovulation, which is caused by the rise of progesterone. The basal body temperature, which is measured orally or vaginally, was the standard method in scientific circles for timing ovulation before the advent of hormone assays.

The basal body temperature has been used extensively for natural family planning. In this context, it can be concluded that the fertile phase has ended and the late safe days have begun when the basal body temperature has been elevated for three consecutive days. The 3-day rule allows for possible errors. The accuracy of the measurement has been increased by the use of electronic thermometers, but even so the error of the method is considerable.[4] Moreover, in natural family planning, basal body temperature has little value in determining infertility in abnormal cycles, which are common in times of stress, during breast-feeding, and in the climacteric. Marshall[5] studied 4749 regular cycles in 321 couples who restricted intercourse to the late safe days (last third of the cycle), as determined by basal body temperature, and found a failure rate of 6.6 pregnancies per 100 woman-years.

Cervical mucus. The amount and quality of the mucus produced at the cervix and the appearance of the cervix itself undergo marked changes throughout the ovulatory cycle. Shettles[6] predicted the use of these symptoms for natural family planning, but they were not widely used until Billings[7] showed that women could readily identify these changes simply by noting sensations in the vulval area. Furthermore, Billings showed that the last day of clear, slippery, "fertile-type" mucus resembling raw egg white before the change back to minimal, thick mucus was closely associated with ovulation and was the day of maximum fertility. This was defined as the "peak" day.[7] These observations were tested by extensive laboratory hormone analyses, which showed that the beginning of the mucus symptom coincided with the beginning of estrogen production by the ovaries (on average, 6 days before ovulation), that the symptoms reached a maximum on the day of the preovulatory estrogen peak, that the peak day occurred within ± 1 day of ovulation in 78% of cycles (n = 179), and that the rapid return to "infertile-type" mucus was caused by the rapid postovulatory rise in progesterone.[8-10] Rules were formulated to apply these findings to the identification of both the early and late safe days of the cycle for natural family planning in the complete range of normal and abnormal cycles. This method of natural family planning, which depends solely on the mucus symptoms, is known as the ovulation method (Billings) and is used worldwide.

To use the ovulation method, couples require adequate training. The World Health Organization has performed a prospective trial of this method in five countries (New Zealand, Ireland, the Philippines, India, and El Salvador) to assess its acceptability and effectiveness. The teaching phase involved 884 couples for three cycles each, and the application phase included 725 couples for a total of 7514 cycles. Ninety-three percent of the women could be taught to recognize and record their symptoms within three cycles; the average number of days per month required for abstinence was 17. The discontinuation rate after 13 cycles was 36%, 20% of which was because of pregnancy. The pregnancy rates per 100 woman-years were 15% as a result of conscious departure from the rules,

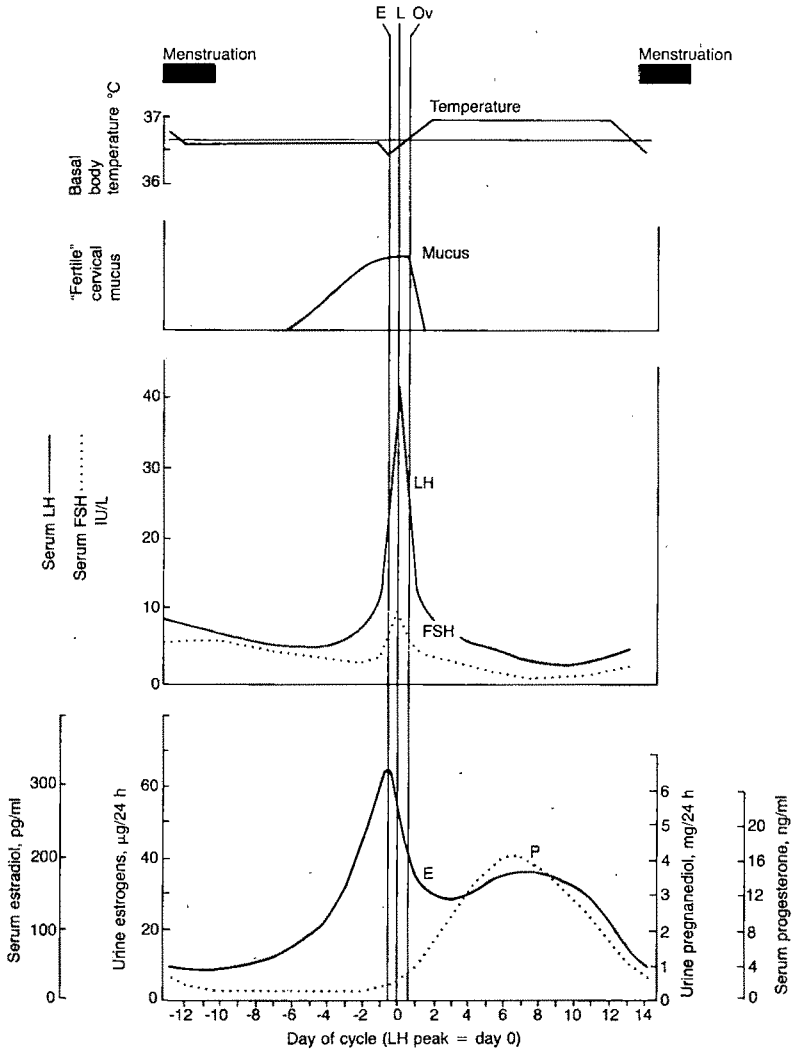


Fig. 2. Mean values for serum luteinizing hormone *(LH)*, follicle-stimulating hormone *(FSH)*, estradiol, progesterone, and urinary estrogens, and pregnanediol throughout the menstrual cycle. Basal body temperatures and times of mucus production and menstruation are shown. The *vertical lines* represent the times of preovulatory estrogen peak *(E)*, LH peak *(L)*, and ovulation *(Ov)*.

3.5% as a result of inaccurate application of instructions, and 3.0% as a result of actual method failure. The rate of pregnancy resulting from method failure varied from 9.4 per 100 woman-years in New Zealand to 5.1 in Ireland, to 1.1 in the Philippines, to 0 in India and El Salvador.[11] The results provided satisfaction for all parties, whatever their point of view.

The outcome of the World Health Organization trial highlights the difficulties involved in comparing natural family planning with other methods of family planning. Natural family planning provides couples with information and not contraception. The contraceptive efficacy of the method depends on how the couples apply the information and on whether they change their minds at any time.

The symptothermal variants of the ovulation method add basal body temperature and the rhythm calculations. However, it is unlikely that further major breakthroughs in natural family planning will result from refinements of the symptoms alone.

Hormone assays

We have investigated the use of home hormone analyses for natural family planning. During the validation of the ovulation method, we found that the women used the results of our urinary estrogen and pregnanediol assays for natural family planning in preference to using their symptoms. These assays depend on spectrophotofluorimetry and gas-liquid chromatography and are still our reference methods.[12, 13] We therefore embarked on the development of simpler analytic methods, with the aim of using the urinary estrogen assay

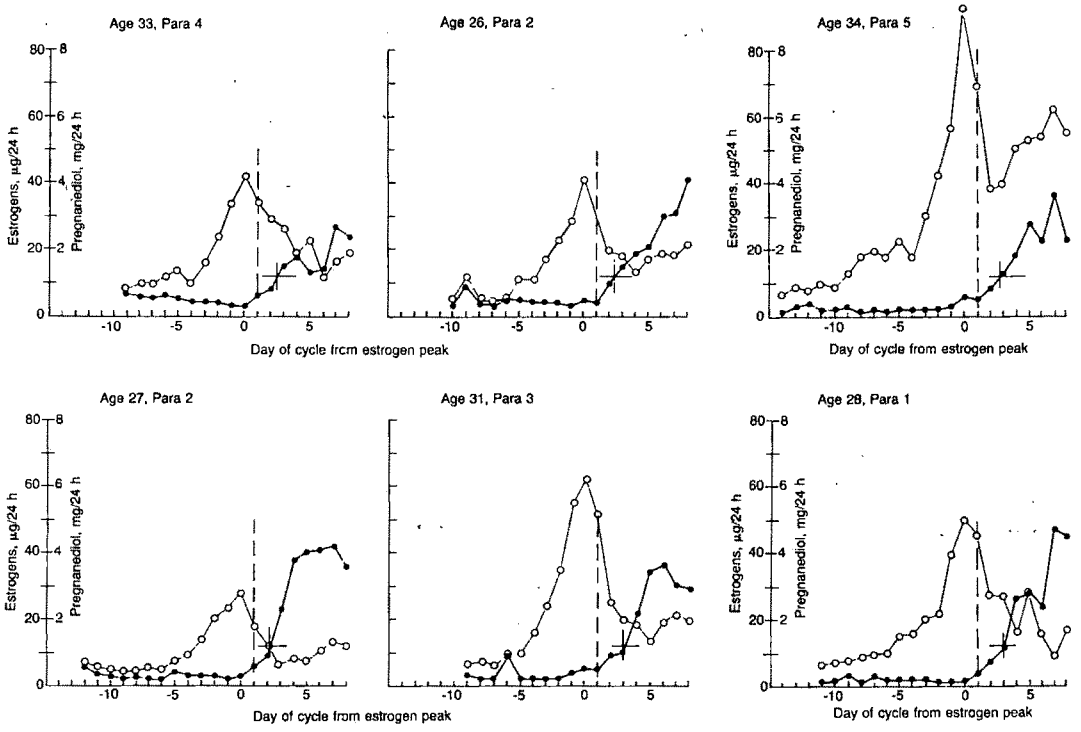


Fig. 3. Six individual cycles showing urinary estrogen and pregnanediol values. Day of the preovulatory estrogen peak = day 0. The *vertical dotted line* is the estimated day of ovulation. The point at which the pregnanediol values reached 1.2 mg/24 hr is marked with a cross.

to determine the beginning of the fertile phase and the pregnanediol assay to signal its end (Figs. 1 and 2). The base data for this development are shown in Fig. 3 for six cycles. Many more examples could be shown to illustrate the points.

The development of suitable home assays was a formidable task for several reasons: (1) These assays must be simple and rapid enough to be performed at home; (2) they must be as reliable and accurate as the best laboratory assays available, since errors will lead to unplanned pregnancies (this is a far more exacting requirement than that needed for the assessment of infertile patients); (3) timed specimens of urine collected overnight, or at least for a 3-hour period, are necessary to achieve the required daily accuracy independent of fluid intake; (4) no noxious chemicals can be used; and (5) the assays must be inexpensive (less than 10 cents each) and easily produced in large numbers.

All these requirements have largely been achieved. We used the system of homogeneous enzyme immunoassay using lysozyme conjugates[14] and measured the urinary estrogen and progesterone metabolites as their glucuronides.[15] We developed methods for both laboratory (wet chemistry) and home use, in which the antisera, conjugates, and substrate are applied to the walls of glass tubes (eye test) or plastic colorimeter cells (photometer test). In the home version, all that is necessary is the accurate addition of urine (diluted to 125 ml/hr with tap water) and water and final mixing. The enzyme reaction is measured by the change in transmission over time either by eye or by a simple regulated temperature photometer with an internal timer.

Measurement of urinary pregnanediol glucuronide

The eye test uses a standard tube set to have an enzyme rate equivalent to 5.3 µmol pregnanediol/24 hr (1.2 mg/24 hr); this controls for time and temperature. The reaction takes about 10 minutes, depending on the room temperature, and the results of the test are recorded as very negative, negative, plus-minus, positive, or very positive, relative to the clearing rate of the standard. This test has been used in the home by 32 women who were selected because they had difficulty in recognizing their symptoms or needed absolute contraception for medical reasons. A total of 184 cycles was studied. The tests were performed by the women or their husbands at home, and the results were checked in the laboratory by both the eye test and quantitative assay.

Fig. 4 summarizes the results obtained with the eye test performed in the laboratory compared with the true values obtained by quantitative assay. Plus-minus values, which should have been 5.2 µmol/24 hr,

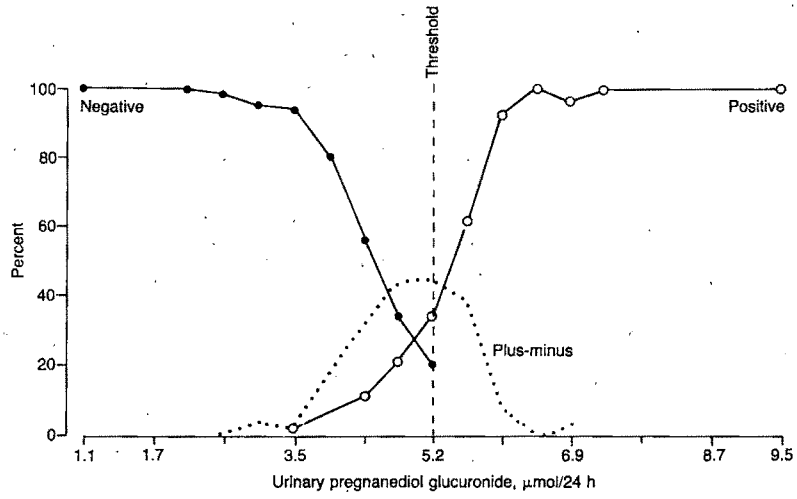


Fig. 4. Percentage of positive, negative, and plus-minus results obtained at each pregnanediol glucuronide level when using the eye test in the laboratory (184 cycles). (Reproduced from Bachelor of Medical Science thesis, Monash University, Melbourne, Australia, 1986 with permission of the author, B. Conway.)

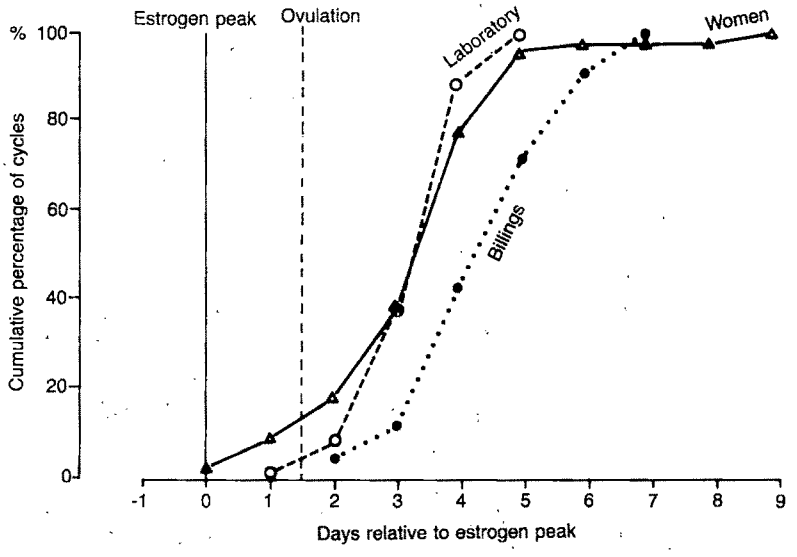


Fig. 5. Cumulative percentage of cycles shown in terms of the day when the pregnanediol eye test (performed at home and in the laboratory) and the mucus symptoms allowed resumption of intercourse (44 cycles). (Reproduced from Bachelor of Medical Science thesis, Monash University, Melbourne, Australia, 1986 with permission of the author, B. Conway.)

were obtained over the actual range of 3.5 to 6.5 μmol/24 hr. This illustrates the remarkable ability of the eye to distinguish between relatively small changes in pregnanediol concentration over the crucial range of interest. As might be expected, the women at home did not do as well, but the results yielded important information on how lay persons cope with scientific analyses and interpret the results. The rule was formulated that a plus-minus value followed by a clear positive or a positive value followed by a more positive value should be obtained before the resumption of intercourse. The results shown in Fig. 5 were obtained. The women using the eye test at home gained one more safe day than they would have with the mucus symptoms method, and none conceived with this rule.

The meter version of the home pregnanediol test has been tested only in the laboratory because of the shortage of photometers. The wet chemistry version of this assay has been compared with the reference laboratory method (x) by means of gas-liquid chromatography and corrected to 100% recovery. The correlation coefficient r was 0.96, and the regression line was Y = 1.13x +

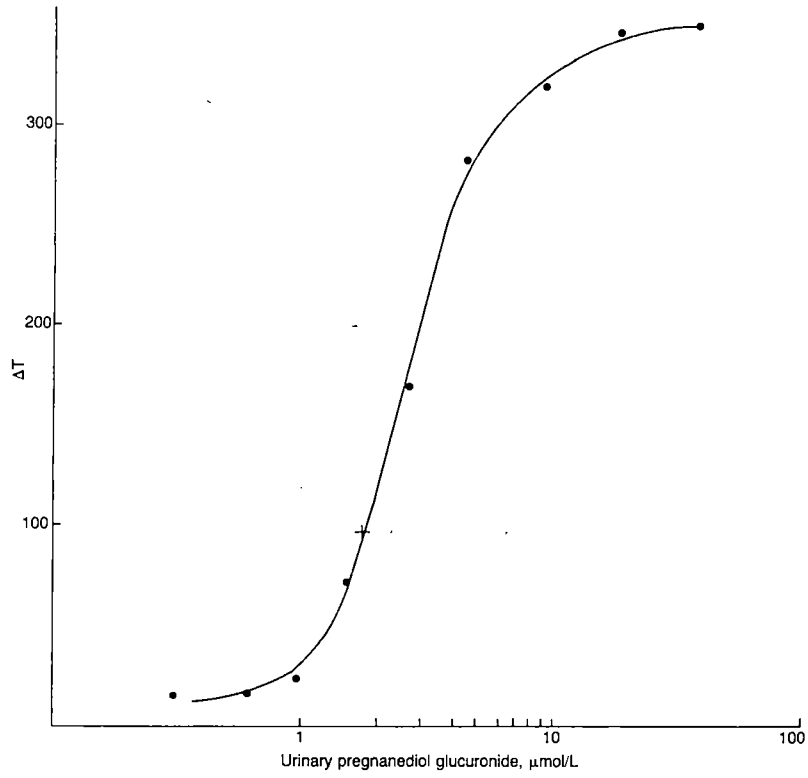


Fig. 6. Standard curve for urinary pregnanediol glucuronide (enzyme immunoassay).

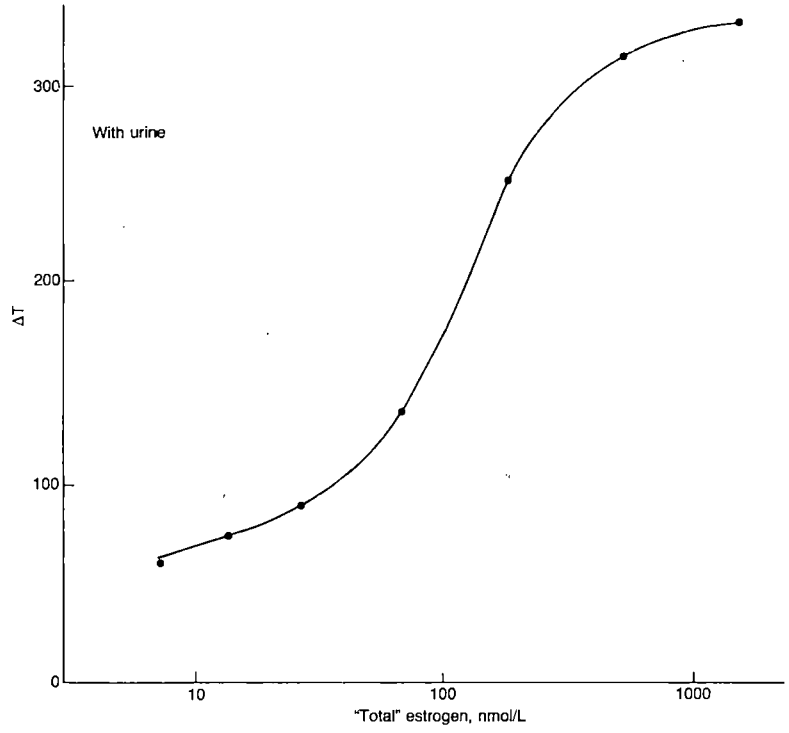


Fig. 7. Standard curve for urinary estrogen glucuronides (enzyme immunoassay).

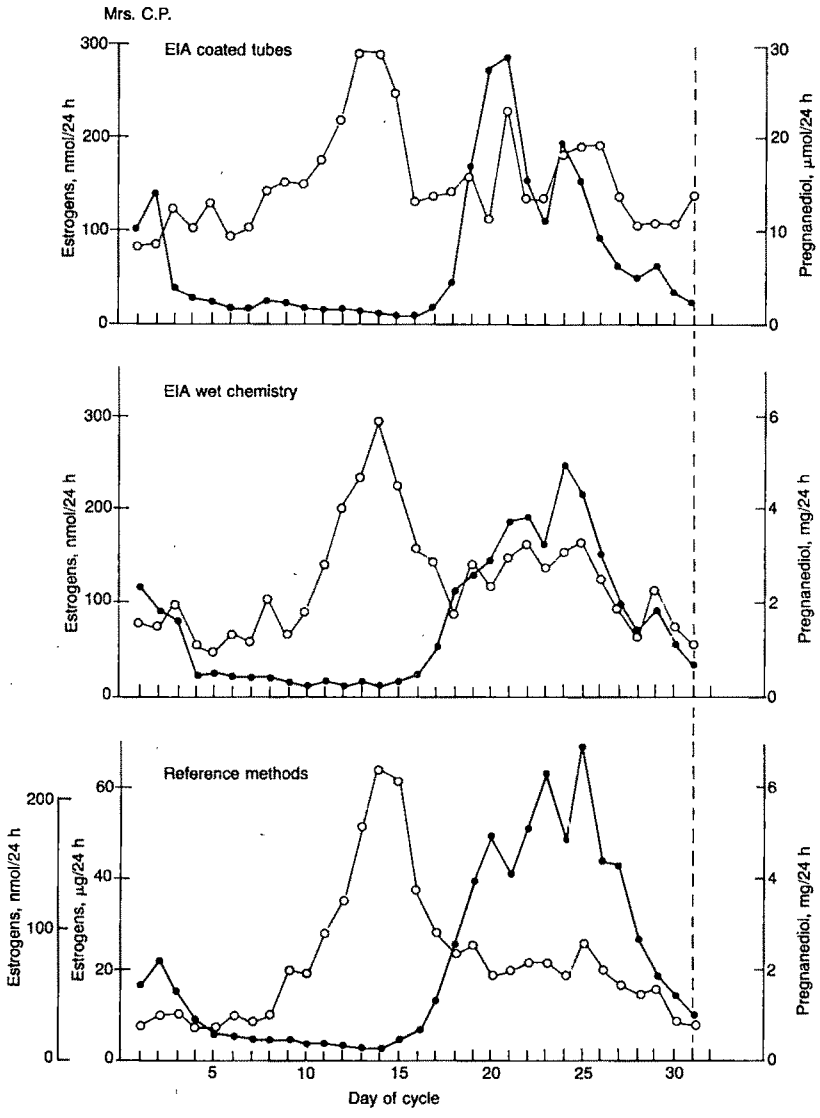


Fig. 8. Preliminary results from a normal cycle showing urinary estrogens measured by spectrophotofluorimetry (reference method) and enzyme immunoassay and urinary pregnanediol measured by gas-liquid chromatography (reference method) and enzyme immunoassay (both wet chemistry and coated tubes). Quantitative differences between methods are mainly due to whether allowance has been made for losses occurring in the reference (extraction) methods. The estrogen enzyme immunoassay shows a urine bias that results in higher baseline values than those given by the reference method.

0.01 µmol/24 hr (n = 65). The slope 1.13x is consistent with the known presence of irremovable water of crystalization in the pregnanediol glucuronide used for standards. The enzyme immunoassay for pregnanediol glucuronide has been extensively validated, and as a 30-second wet chemistry procedure, has been used routinely for 3 years in our laboratory. A standard curve for urinary pregnanediol glucuronide is shown in Fig. 6. The reliability criteria of the home meter assay, which uses a reaction time of 5 minutes, are currently being evaluated. It gives the same results as the wet chemistry: Y = 0.99x − 0.07 µmol/24 hr (n = 22); the precision depends on the accuracy of adding the components to the coated tubes and is continually being improved.

Measurement of urinary estrogen glucuronides

The development of an enzyme immunoassay for estrogen has been more difficult because the amounts being measured are much smaller. Nevertheless, a working assay is now being evaluated. The assay requires a photometer, and the reaction time is 20 minutes. It is available as wet chemistry for laboratory use and coated tubes for home use. The assay measures a

mixture of estrone-glucuronide, estriol-3-glucuronide, and estriol-16-glucuronide.

A standard curve for urinary estrogen glucuronides is shown in Fig. 7. Application to a complete menstrual cycle is shown in Fig. 8, together with the results obtained by the reference estrogen method and the pregnanediol methods of enzyme immunoassay and gas-liquid chromatography. The early estrogen rise and the preovulatory estrogen peak are readily identified. When the first production run of photometers is finished shortly, these methods will be completely evaluated for home, medical center, and laboratory use and will then be made available for distribution.

Discussion

In summary, we can state that women can collect timed specimens of urine and perform pregnanediol analyses at home with competence. The corresponding estrogen assay is ready for similar testing. Application of these analyses to large populations of women practicing natural family planning will greatly increase our knowledge of human reproduction in all its aspects. Their full value in the teaching and practice of natural family planning and in the assessment of infertility has yet to be determined. Furthermore, having unambiguous identification of the day of ovulation in unplanned pregnancies will make it possible to determine exactly what went wrong. This will surely help increase the effectiveness of natural family planning in the same way that in-flight black box recorders have increased the safety of air travel.

These methods may seem to be too complicated to be performed and interpreted by most women at home. However, they involve less labor and cost than does making a cup of tea. In interpreting these assays, the patterns obtained are as important as the absolute amounts. A woman starts early in her cycle by measuring urinary estrogens. She will first see baseline values, followed by a rise, which indicates the beginning of fertility. When this rise is clearly established or a fall occurs, she changes to measuring pregnanediol. When postovulatory pregnanediol values are clearly reached (5.2 μmol/24 hr), she knows that the late safe days have started and that no further testing is required for that cycle. This represents a total of approximately 12 days of testing. It remains to be seen whether widespread education will lead to a general application of this aid to natural family planning.

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A new delivery system for contraceptive steroids

Sheldon Segal, Ph.D.
New York, New York

Long-acting forms of contraception that take advantage of the tissue compatibility of silicone rubber, a polymer of dimethylsiloxane, have been developed. The most advanced type of subdermal contraceptive capsule contains about 36 to 40 mg of levonorgestrel and is 30 mm in length and 2.4 mm in diameter (Norplant). The set of six implants is placed under the skin of the upper arm with a 10-gauge trocar with the use of a local anesthetic. The basis for the antifertility effect of these implants is continuous, low-dose progestogen therapy without the use of estrogen. The capsules release a total of approximately 39 μg of levonorgestrel each day. The blood progestogen level achieved through this microabsorption delivery system is 0.25 to 0.3 ng/ml. There is a sufficient supply of steroid in the capsules to maintain this blood progestogen level for 5 to 6 years. Since the capsules can be removed at any time, this contraceptive method is voluntarily reversible. The effectiveness of these subdermal implants has been evaluated in long-term studies that have progressed for well over 5 years. Therefore one clinic visit eliminates nearly 2000 days of concern over having to remember to take a pill on schedule. There has been sufficient experience to assure that fertility recurs promptly after removal of the subdermal implants. (Am J Obstet Gynecol 1987;157:1090-2.)

Key words: Contraception, long-acting subdermal implants, levonorgestrel

Many new delivery systems are under investigation in the field of contraception. Among these are systems that use silicone rubber, a polymer of dimethylsiloxane, as a carrier for the slow release of contraceptive progestogen.

Evolution of subdermal contraceptive implants

The development of a long-acting form of contraception that takes advantage of the tissue compatibility of silicone rubber began in our laboratories some 20 years ago with the demonstration that many steroid substances can be released from silicone rubber at a slow and steady rate. Lipophilic compounds, such as steroids, are soluble in and pass through a solid matrix of polydimethylsiloxane. Thus a supply of crystalline steroid stored within a silicone rubber capsule will pass through the polymer wall at a controlled rate. The steroid can also be released from a solid rod composed of a mixture of liquid polymer and crystalline steroid, which is homogenized and then prompted to solidify by adding an appropriate catalyst. Silicone rubber was also deemed suitable as a basis for a contraceptive modality because of its record of medical safety. Silicone rubber tubing has been used for subdermal hydrocephalus drains, and a medical-grade preparation of the polymer is used in various reconstructive or cosmetic surgical procedures.

By 1968 the first clinical trials had begun, and 3 years later, a series of studies were undertaken to choose the best progestogen candidate for use in a long-acting, low-dose, continuous administration contraceptive. Levonorgestrel was selected as the compound of choice for the first subdermal contraceptive implant system because it is released at the extremely slow rate of about 10% of the original load per annum. This means that it would take about 5 years for 50% of the original dose of levonorgestrel to be released, which portended a very long duration of contraceptive efficacy.

The subdermal contraceptive system of Norplant has

Table I. Plasma levonorgestrel levels with Norplant

Mo of treatment	*No. of women*	*LNG, ng/ml (mean)*	*SD*	*p*
1-12	27	0.35	0.13	
13-24	23	0.31	0.11	NS
25-36	30	0.34	0.11	NS
37-48	8	0.32	0.08	NS
49-60	42	0.29	0.11	<0.05

LNG = Levonorgestrel.

Table II. Five-year cumulative study of Norplant efficacy

Yrs of use	*Pregnancies*	*Woman-years*	*Rate per 100*
1	3	894	0.3
2	4	1459	0.3
3	6	1878	0.3
4	10	2187	0.5
5	11	2380	0.5

From Population Sciences, The Rockefeller Foundation.

Table III. Norplant net cumulative continuation rates per 100 acceptors (N = 992)

	Rate or No.				
	12 mo	*24 mo*	*36 mo*	*48 mo*	*60 mo*
Pregnancy	0.3	0.5	0.7	0.7	0.7
Menstrual problems	8.6	12.9	14.4	15.2	16.6
Medical	4.7	8.2	10.2	12.0	12.8
Continuation	79.4	58.3	49.0	43.2	39.3
No. at risk in month	787	331	174	92	51
Woman-months of use	10,601	16,334	18,785	20,147	20,823

Table IV. Rate of discontinuation of Norplant because of menstrual disturbances

	Cumulative termination rate/100 acceptors		
Menstrual disturbance	*Yr 1*	*Yr 2*	*Yr 3*
Frequent, irregular bleeding	4.9	6.6	7.1
Heavy menstrual flow	0.5	1.4	1.9
Prolonged bleeding	2.8	4.1	4.8
Dysmenorrhea	0.0	0.0	0.0
Amenorrhea	0.7	0.9	0.9
Spotting	0.5	0.8	0.8
Other bleeding	0.0	0.2	0.5
Total	9.5	13.9	16.0

Table V. Restoration of fertility after removal of Norplant implants

	% of women who became pregnant after discontinuing of contraception	
Mo	*Norplant system*	*TCu 200*
1	21.6	11.3
3	43.1	33.0
6	67.3	47.8
12	76.6	73.9
No. of women	53	29

Table VI. First-year results of field studies of effectiveness and continuation of Norplant implants

Country	*Pregnancy rates*	*Continuation*	*n*
Colombia	0.0	93.0	300
Ecuador	0.0	87.4	283
Egypt	0.8	89.6	250
Indonesia	0.5	95.3	813
Thailand	0.3	88.4	704

been developed as a hollow capsule 30 mm in length and 2.4 mm in diameter filled with about 36 to 40 mg of crystalline levonorgestrel. The capsules are placed under the skin of the upper arm with a 10-gauge trocar with the use of a local anesthetic. The steroid is released at a slow, controlled rate of about 6 μg per capsule per day. Therefore a set of six implants results in a release rate of about 36 to 40 μg/day, which is sufficient to achieve an antifertility effect. A second system, Norplant-2, has also been developed and consists of a solid rod of homogenized 50% silicone rubber–50% steroid mixture covered with ultrathin silicone rubber tubing to hold the entire rod in place as steroid dissolution proceeds.

Efficacy and safety

The effectiveness of Norplant has been evaluated in long-term studies that have progressed for well over 5 years. Table I shows that plasma levonorgestrel remains at a relatively constant level approximating 0.3 ng/ml over a 5-year period, declining just slightly in the fifth year. Plasma levonorgestrel levels high enough to protect against pregnancy are maintained 2 to 3 years beyond the recommended 5-year period of effectiveness. This may be potentially important should the user delay returning for a replacement set of implants.

In one 5-year study, only 11 pregnancies occurred in 2380 woman-years of use, which translates into a pregnancy rate well under 1 pregnancy per 100 woman-years of use (Table II). Life-table analysis of another 5-year study involving nearly 1000 women indicates that the level of protection against pregnancy year by year approaches the effectiveness of surgical contraception. In this clinical trial, the 50% discontinuation of use rate was reached at about 3 years (Table III).

The main reason for discontinuation of Norplant is menstrual problems, most commonly frequent irregular bleeding (Table IV). About 5% of women discontinue the method for this reason during the first year of use, an additional 1.5% do so during the second year, and an additional 1% or less during the third year. The total discontinuation rate from bleeding-related problems after 3 years is 15.1% (Table IV). Amenorrhea is rarely a cause for discontinuation.

Extensive safety studies carried out with Norplant indicate no unfavorable changes in liver function, lipid metabolism, or blood pressure, no increase in the risk

of ectopic pregnancy, and no increase in total menstrual blood loss. In fact, Norplant tends to increase hemoglobin levels in women who had low levels before using the implants.

Since the implants can be removed at any time, this contraceptive method is voluntarily reversible, and there has been sufficient experience to ensure that fertility recurs promptly after discontinuation. According to one study of women who discontinued the use of either Norplant or an intrauterine device because they wished to become pregnant, approximately three quarters of women in both groups became pregnant within 1 year (Table V).

Much of the data discussed here comes from studies of the 6-capsule Norplant system. However, clinical trials of the Norplant-2 system show essentially the same level of performance over a 3-year period of observation.

Norplant has been approved for general use in Finland, Sweden, Thailand, Indonesia, and the Dominican Republic and is being tested in 31 additional countries, including the United States. First-year results from field studies in some countries confirm the very high level of effectiveness that had been observed in the clinical trials (Table VI). The continuation rates are even higher than those reported in the clinical trials and range around 90% after the first year of use. This generally favorable experience has continued in years 2, 3, and even 4 in some of these field trials.

In summary, the high efficacy of Norplant makes this contraceptive method an important reversible alternative to sterilization. It affords long-acting protection, which means that one visit to the clinic can replace virtually thousands of days of pill taking. Norplant can be used for birth spacing because the implants can be removed at any time and fertility restored without impairment. It is also a suitable option for immediate postabortal use and when intrauterine devices or estrogens are contraindicated. New routes of administration should thus prove to be an important adjunct to existing modalities for effective and reversible contraception.

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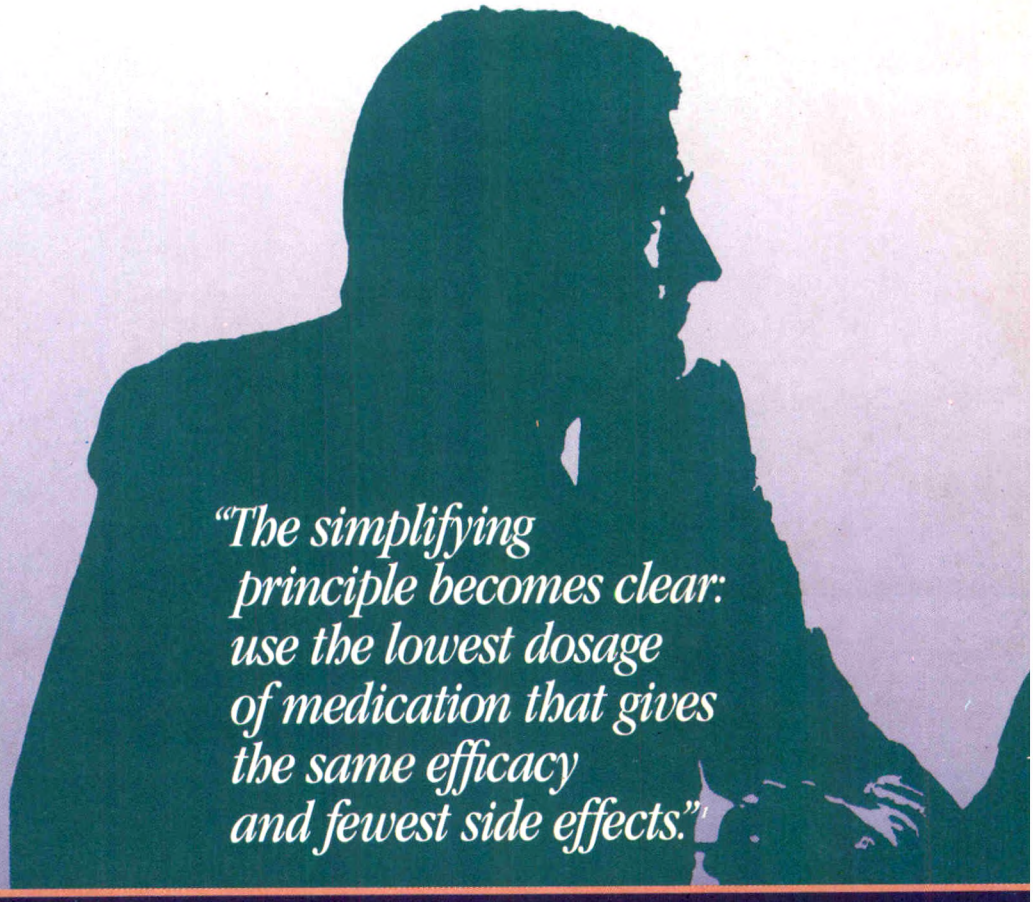
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Opinions from three of

Today's authoritie



"The simplifying principle becomes clear: use the lowest dosage of medication that gives the same efficacy and fewest side effects."[1]

Low Dose with Tri-Norinyl

- 29% less progestin per cycle than a 1/35 formulation[2*]

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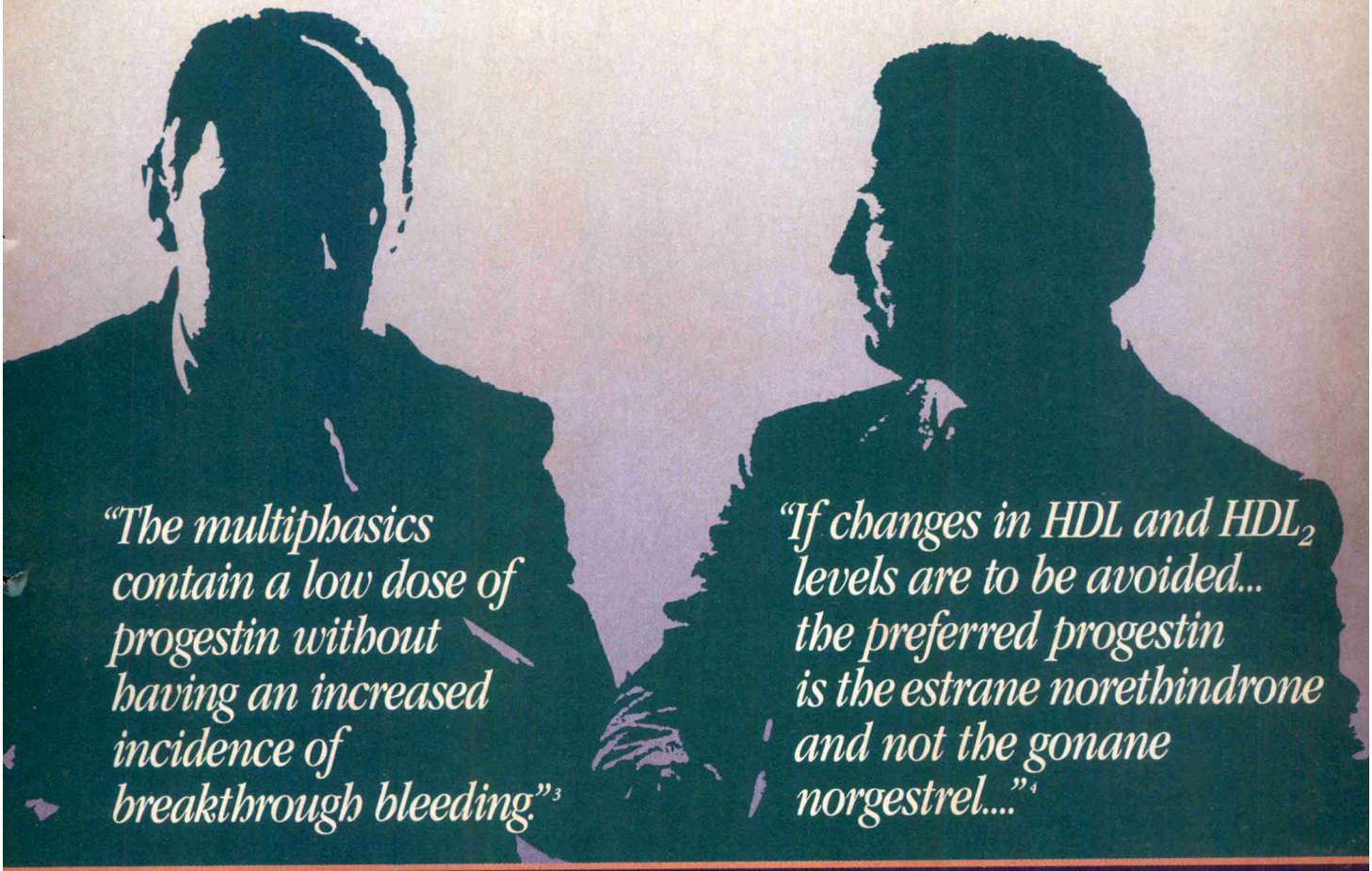
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*1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Comparative data available from Syntex Laboratories, Inc.

No endorsement of Tri-Norinyl or any other Syntex oral contraceptive has been given by the authorities cited.

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on oral contraception:



"The multiphasics contain a low dose of progestin without having an increased incidence of breakthrough bleeding."[3]

"If changes in HDL and HDL_2 levels are to be avoided... the preferred progestin is the estrane norethindrone and not the gonane norgestrel...."[4]

Low BTB with Tri-Norinyl

- Extra midcycle protection that's unique among multiphasics
- Low BTB rates—equivalent to a fixed low-dose OC[2]

Low Impact with Tri-Norinyl

- Minimal lipid and cholesterol impact[4]
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THE LOWEST-DOSE MULTIPHASIC OC WITH NORETHINDRONE

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of prescribing information on next page.

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TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34 – 5/100,000; ages 35-44 – 33/100,000; ages 45-49 – 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 – 1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over – 1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS – RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. (See also Warnings, 4. *Liver Tumors.*) 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use; risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. Cases of hepatocellular carcinoma have been reported in long-term users of OCs.[105-107] An epidemiological study suggested a 4-fold increase in relative risk for hepatocellular carcinoma in OC users for 8 years or more, which increased to 7 in women with markers of hepatitis B. A second study suggested a risk of about 20 for use in excess of 5 years. 5. *Use in or Immediately Preceding Pregnancy; Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones – estrogenic and progestational agents – during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero.* These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use acequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests that the incidence of *Chlamydia trachomatis* infection, the leading cause of nongonococcal PI.D., is increased by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Budd-Chiari syndrome, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure, premature supraventricular contractions. **Information for the Patient** (See Patient Package Insert).

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WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

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Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

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Contents

November
1987

CLINICAL SECTION

Clinical Opinion

Stress in residency: Proposals for solution 1093
Preston V. Dilts, Jr., M.D., and Stephen L. Dilts, M.D., Ph.D.
Ann Arbor, Michigan, and Denver, Colorado

The pregnant brain dead and the fetus: Must we always try to wrest life from death? 1097
Erich H. Loewy, M.D.
Peoria and Chicago, Illinois

Clinical Articles

Unintended laparotomy associated with laparoscopic tubal sterilization 1102
Adele L. Franks, M.D., Juliette S. Kendrick, M.D., and Herbert B. Peterson, M.D.
Atlanta, Georgia

(Contents continued on page 7A)

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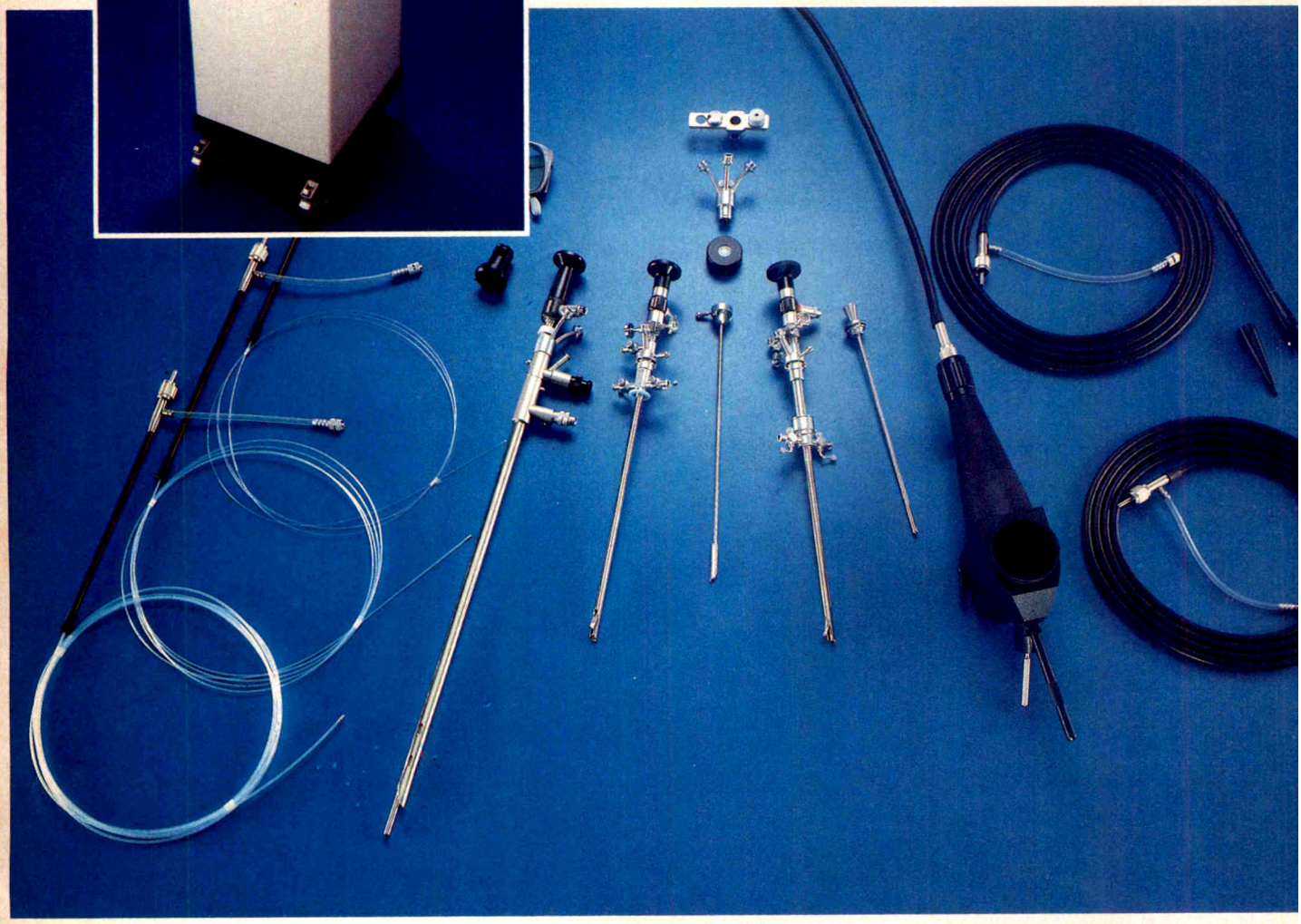
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Contents continued from page 5A

Decreasing incidence of invasive cervical cancer in young women 1105
Joseph Chu, M.D., M.P.H., and Emily White, Ph.D.
Seattle, Washingon

Menstrual and reproductive factors and the risk of myocardial infarction in women under fifty-five years of age 1108
Carlo La Vecchia, M.D., Adriano Decarli, Sc.D., Silvia Franceschi, M.D., Antonella Gentile, Ph.D., Eva Negri, Sc.D., and Fabio Parazzini, M.D.
Milan, Italy

The "breakfast tolerance test": Screening for gestational diabetes with a standardized mixed nutrient meal 1113
Donald R. Coustan, M.D., John A. Widness, M.D., Marshall W. Carpenter, M.D., Lee Rotondo, R.N., and Doreen Chin Pratt, R.D.
Providence, Rhode Island

Gestational diabetes: Maternal response to diet and insulin therapy as reflected by glycosylated hemoglobin concentration 1118
Arnold S. Grandis, M.D., Ph.D., Mary Ann Morris, M.D., and Jean C. Litton, R.N., B.S.
Durham, North Carolina

Preliminary observations on lactoferrin secretion in human vaginal mucus: Variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae* 1122
Myron S. Cohen, M.D., Bradley E. Britigan, M.D., Martha French, B.S., and Karen Bean, B.S.
Chapel Hill, North Carolina

Human chorionic gonadotropin, estriol, and testosterone changes in two pregnancies with hyperreactio luteinalis 1126
Eberhard K. Muechler, M.D., Jeffrey Fichter, M.D., and John Zongrone, B.S.
Rochester, New York

Carbon dioxide laser laparoscopy performed with a flexible fiber in humans 1129
Michael S. Baggish, M.D., Pavlos Baltoyannis, M.D., Shawky Badawy, M.D., and Doris Laurey, R.N.
Syracuse, New York

Decreased elastic fibers and desmosine content in incompetent cervix 1134
Phyllis C. Leppert, M.D., Ph.D., Shiu Yeh Yu, Ph.D., Stephen Keller, Ph.D., Joseph Cerreta, Ph.D., and Ines Mandl, Ph.D.
New York and Jamaica, New York, and St. Louis, Missouri

(Contents continued on page 10A)

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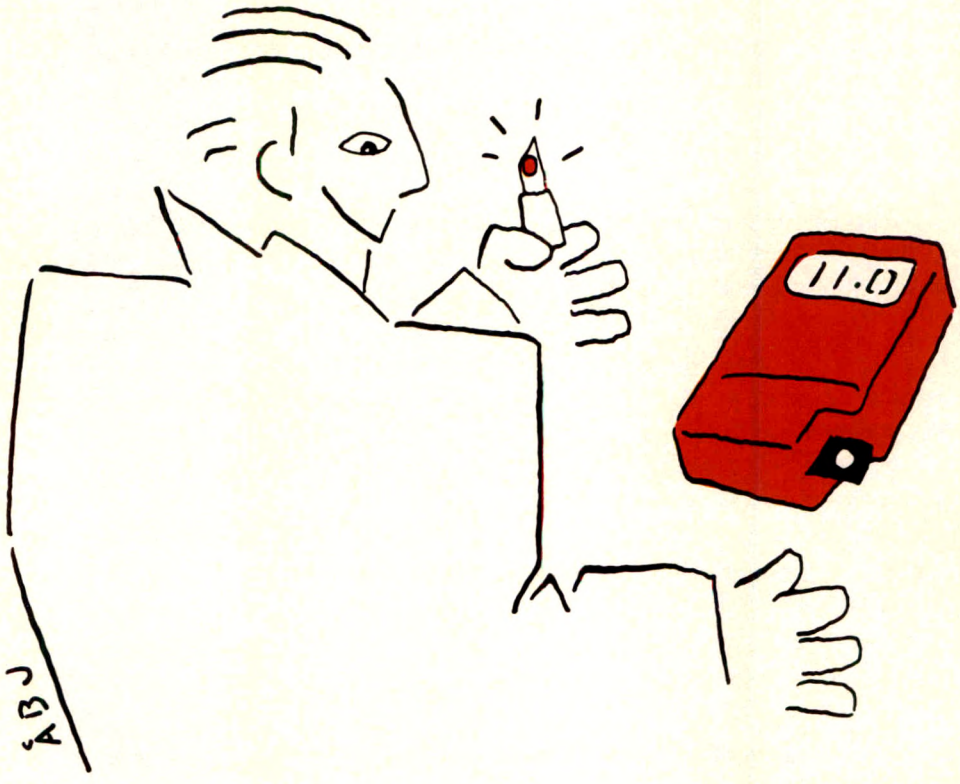
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Contents continued from page 7A

Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes 1140
Norio Shinozuka, M.D., Takashi Okai, M.D., Shiro Kohzuma, M.D., Masaaki Mukubo, M.D., Chen-Ting Shih, M.D., Tsugio Maeda, M.D., Yoshinori Kuwabara, M.D., and Masahiko Mizuno, M.D.
Hongo, Bunkyo-ku, Tokyo, Japan

Soft tissue nuchal fold in the second-trimester fetus: Standards for normal measurements compared with those in Down syndrome 1146
Beryl R. Benacerraf, M.D., and Fredric D. Frigoletto, Jr., M.D.
Boston, Massachusetts

First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions 1150
Barbara A. Strobino, Ph.D., and Judy Pantel-Silverman, M.P.H.
New York, New York

Emotional responses of pregnant women to chorionic villi sampling or amniocentesis 1155
John W. Spencer, Ph.D., and David N. Cox, Ph.D.
Burnaby, British Columbia, Canada

Hereditary protein C deficiency during pregnancy 1160
Benjamin Brenner, M.D., Amos Shapira, M.D., Charles Bahari, M.D., Luis Haimovich, M.D., and Uri Seligsohn, M.D.
Tel-Aviv, Israel

A very rare fetal malformation: The cutaneous widespread vascular hamartomatosis 1162
Riccardo Arienzo, M.D., Cosimo S. Ricco, M.D., and Filomena Romeo, M.D.
Naples, Italy

Updated pregnancy rates for the Today contraceptive sponge 1164
David A. Edelman, Ph.D., and Barbara B. North, Ph.D., M.D.
Chapel Hill, North Carolina, and Laguna Niguel, California

Treatment of nonmetastatic gestational trophoblastic disease with oral methotrexate 1166
James F. Barter, M.D., Seng Jaw Soong, Ph.D., Kenneth D. Hatch, M.D., James W. Orr, Jr., M.D., Edward C. Partridge, M.D., J. Maxwell Austin, Jr., M.D., and Hugh M. Shingleton, M.D.
Birmingham, Alabama

Sonographic diagnosis of a bladder polyp 1168
Lars P. Jensen, M.D., and Charles M. Lynne, M.D.
Miami, Florida

Contents

Sonographic evaluation of the cervix during ovulation induction 1170
Lyndon M. Hill, M.D., Carolyn B. Coulam, M.D., Sandra L. Kislak, R.T., R.D.M.S., Carol S. Peterson, R.D.M.S., and Cynthia J. Runco, R.D.M.S.
Pittsburgh, Pennsylvania

A prospective randomized study of the aggressive management of early labor 1174
Gary R. Cohen, M.D., William F. O'Brien, M.D., Lloyd Lewis, M.D., and Robert A. Knuppel, M.D., M.P.H.
Tampa, Florida

Breast stimulation test and oxytocin challenge test in fetal surveillance: A prospective randomized study 1178
Shlomo Lipitz, M.D., Gad Barkai, M.D., Jaron Rabinovici, M.D., and Shlomo Mashiach, M.D.
Tel Hashomer, Israel

Intrapartum use of fetal scalp hematocrit in the diagnosis of profound fetal anemia caused by fetomaternal hemorrhage 1182
Arie Herman, M.D., Yan Bukovsky, M.D., Levi Benson, M.D., Zvi Weinraub, M.D., and Eliyahu Caspi, M.D.
Zerifin, Israel

Prevention of pelvic adhesion information by different modalities of treatment 1184
Jamil A. Fayez, M.D., and Peter J. Schneider, M.D.
Winston-Salem, North Carolina

A thirty-year review of maternal mortality in Oklahoma, 1950 through 1979 1189
Vijayakumar Moses, M.B., B.S., M.D., Sara Reed DePersio, M.D., M.P.H., Dick Lorenz, M.S.P.H., Mark W. Oberle, M.D., M.P.H., Roger Rochat, M.D., and Aurora Fermo, M.D.
Oklahoma City, Oklahoma, and Atlanta, Georgia

Anorexia nervosa, bulimia, and pregnancy 1194
Donna E. Stewart, M.D., Joel Raskin, M.D., Paul E. Garfinkel, M.D., Ophelia L. MacDonald, M.D., and G. Erlick Robinson, M.D.
Toronto, Ontario, Canada

Effects of aerobic and strength conditioning on pregnancy outcomes 1199
Douglas C. Hall, M.D., and David A. Kaufmann, Ph.D.
Ocala and Gainesville, Florida

(Contents continued on page 13A)

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Contents continued from page 11A

Evaluation of a perinatal grief support team 1203
Marian F. Lake, R.N.C., M.P.H., Thomas M. Johnson, Ph.D., Jane Murphy, M.P.A., and Robert A. Knuppel, M.D., M.P.H.
Tampa, Florida

Local peritoneal factors: Their role in infertility associated with endometriosis 1207
Sherif G. Awadalla, M.D., Chad I. Friedman, M.D., Amin U. Haq, M.D., Sung I. Roh, M.D., NeeOo W. Chin, M.D., and Moon H. Kim, M.D.
Columbus, Ohio

The morphologic effect of short-term medical therapy of endometriosis 1215
Ivo A. Brosens, M.D., Ph.D., Ann Verleyen, M.D., and Freddy Cornillie, D.Sc.
Leuven, Belgium

Cord sampling for the evaluation of oxygenation and acid-base balance in growth-retarded human fetuses 1221
Giorgio Pardi, M.D., Mauro Buscaglia, M.D., Enrico Ferrazzi, M.D., Patrizia Bozzetti, M.D., Anna Maria Marconi, M.D., Irene Cetin, M.D., Frederick C. Battaglia, M.D., and Edgar L. Makowski, M.D.
Milan, Italy, and Denver, Colorado

The length of the human umbilical cord in twin pregnancies 1229
Torgrim Soernes, M.D., and Trygve Bakke, M.D.
Norbyhagen, Norway

Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole 1230
Henk C. S. Wallenburg, M.D., Ph.D., and Nel Rotmans, B.Sc.
Rotterdam, The Netherlands

Caffeine-cigarette interaction on fetal growth 1236
Louise Beaulac-Baillargeon, Ph.D., and Carole Desrosiers, B.Sc.(Pharm.), M.Sc.
Ste-Foy, Quebec, Canada

The effect of smoking on placental and fetal zinc status 1241
P. M. Kuhnert, Ph.D., B. R. Kuhnert, Ph.D., P. Erhard, B.S., W. T. Brashear, M.S., S. L. Groh-Wargo, M.S., and S. Webster, M.S.
Cleveland, Ohio

The relationship between cadmium, zinc, and birth weight in pregnant women who smoke 1247
B. R. Kuhnert, Ph.D., P. M. Kuhnert, Ph.D., S. Debanne, Ph.D., and T. G. Williams, M.S.
Cleveland, Ohio

(Contents continued on page 15A)

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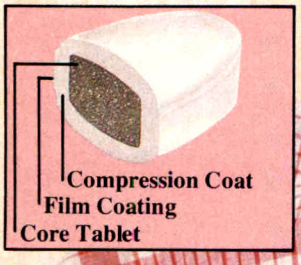
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Vitamin E, IU	30	100
Vitamin C, mg	90	150
Folic Acid, mg	1	125
Thiamine (B_1), mg	2.55	150
Riboflavin (B_2), mg	3	150
Niacin, mg	20	100
Vitamin B_6, mg	10	400
Vitamin B_{12}, mcg	8	100
Biotin, mg	0.05	16
Pantothenic Acid, mg	15	150
MINERALS		
Calcium, mg	200	15
Iodine, mcg	150	100
Iron,mg	60	333
Magnesium, mg	100	22
Copper, mg	2	100
Zinc, mg	15	100
Artificial color		none
Artificial flavor		none

Natalins Rx is a multivitamin, multimineral supplement for pregnant or lactating women. Daily dose: 1 tablet or as prescribed.
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Contents continued from page 13A

Pleural effusions and pulmonary hypoplasia 1252
Ramon A. Castillo, M.D., Lawrence D. Devoe, M.D., Greer Falls, M.D., Gerald B. Holzman, M.D., Hamid A. Hadi, M.D., and Hossam E. Fadel, M.D.
Augusta, Georgia

Antenatal diagnosis and management of monoamniotic twins 1255
John F. Rodis, M.D., Anthony M. Vintzileos, M.D., Winston A. Campbell, M.D., Jeffrey L. Deaton, M.D., Fred Fumia, M.D., and David J. Nochimson, M.D.
Farmington, Connecticut

Blood pressure in the midtrimester and future eclampsia 1258
Leon C. Chesley, Ph.D., and Baha M. Sibai, M.D.
Memphis, Tennessee

Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons 1261
Randy B. Howard, Ph.D., Tomokazu Hosokawa, Ph.D., and M. Helen Maguire, Ph.D.
Kansas City, Kansas

Haptoglobin helps diagnose the HELLP syndrome 1267
Peeter A. Poldre, M.D.
Toronto, Ontario, Canada

Insufficient transplacental digoxin transfer in severe hydrops fetalis 1268
Johnny S. Younis, B.M.Sc., M.D., and Menachem Granat, M.D.
Jerusalem, Israel

Renal arterial blood flow velocity waveforms in pregnant women 1269
Toshiyuki Hata, M.D., Kohkichi Hata, M.D., Showa Aoki, M.D., Osamu Takamiya, M.D., Fuminori Murao, M.D., and Manabu Kitao, M.D.
Izumo, Japan

The endocrinologic evaluation of a 45,X true hermaphrodite 1272
Gregory F. Rosen, M.D., Michael Vermesh, M.D., Gerrit d'Ablaing III, M.D., Stephen Wachtel, Ph.D., and Rogerio A. Lobo, M.D.
Los Angeles, California, and Memphis, Tennessee

Hyperimmunoglobulinemia E and pregnancy: A case report 1273
Connie Lindenbaum, M.D., Ashwin Chatwani, M.D., Rosanne Oyer, M.D., and John Fitzgerald, D.O.
Philadelphia, Pennsylvania

2-Chloroprocaine for local perineal infiltration 1275
Elliot H. Philipson, M.D., Betty R. Kuhnert, Ph.D., and Carol D. Syracuse, B.S.
Cleveland, Ohio

(Contents continued on page 17A)

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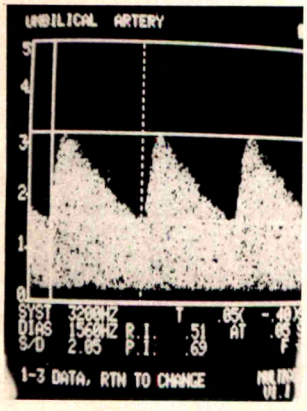


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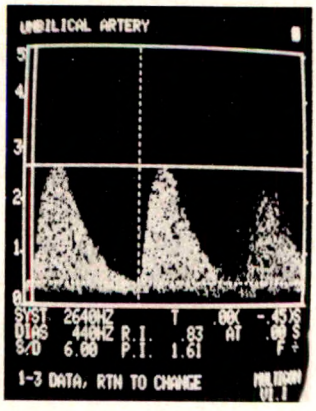
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S/D = 2.05



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(914) 664-7300 Telex 6974662 MULTIGN

Contents continued from page 15A

Breast galactography in the treatment of infected cysts 1278
Dennis P. Tihansky, M.D., Ph.D., and Joseph A. DiBlasio, M.D.
Valhalla, New York, and Clarksburg, West Virginia

BASIC SCIENCE SECTION

Effects of the antiprogesterone RU 486 on preterm birth in the rat 1281
R. E. Garfield, Ph.D., J. M. Gasc, Ph.D., and E. E. Baulieu, M.D.
Hamilton, Ontario, Canada, and Paris, France

Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration 1286
K. Wayne Riggs, M.Sc., Dan W. Rurak, D.Phil., Sun D. Yoo, M.Sc.,
Barbara A. McErlane, B.Sc., Sandra M. Taylor, A.H.T.,
Graham H. McMorland, M.B., Ch.B., and James E. Axelson, Ph.D.
Vancouver, British Columbia, Canada

Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus 1292
Michael G. Ross, M.D., M. Gore Ervin, Ph.D., Robert W. Lam, B.S., Lony Castro, M.D.,
Rosemary D. Leake, M.D., and Delbert A. Fisher, M.D.
Torrance, California

Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys 1297
Reinhart B. Billiar, Ph.D., Donald Richardson, M.D., Robert Schwartz, M.D.,
Barry Posner, M.D., and Brian Little, M.D.
Montreal, Quebec, Canada, Pittsburgh, Pennsylvania, and Providence, Rhode Island

Current Development

A new look at uterine muscle contraction 1303
Mary E. Carsten, Ph.D., and Jordan D. Miller, M.D.
Los Angeles, California

CORRESPONDENCE

The detection of facial anomalies with ultrasound William L. Koontz, M.D. *Louisville, Kentucky* 1316

Reply Gianluigi Pilu, M.D., and Luciano Bovicelli, M.D. *Bologna, Italy* 1316

Amniotic fluid trypsin and premature rupture of membranes James A. McGregor, M.D.C.M., Janice I. French, C.N.M., and David Lawellin, Ph.D. *Denver, Colorado* 1316

Reply Naohiro Kanayama, M.D. *Hamamatsu, Japan* 1318

(Contents continued on page 19A)

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Contents continued from page 17A

Possible vascular disruptive origin of hemifacial microsomia? A. Schinzel, M.D. *Zurich, Switzerland* 1319

Placental bed biopsy technique and vascular lesions Carlos A. Labarrere, M.D. *Buenos Aires, Argentina* 1320

Reply W. B. Robertson, M.D. *London, England* 1322

Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia M. C. Anderson, M.D. *London, England* 1322

Reply Michael S. Baggish, M.D. *Syracuse, New York* 1323

Conjoined twins in triplet pregnancy E. Shalev, M.D., and H. Zuckerman, M.D. *Afula, Israel* 1323

ITEMS

Items 1324

CORRECTIONS

Effects of maternal anemia on cardiac output, systemic oxygen consumption, and regional blood flow in pregnant sheep (Edelstone DI, Paulone ME, Maljovec JJ, Hagberg M. 1987;156:740-8) 1291

***Echocardiographic* and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy (Hustin J, Schaaps J-P. 1987;157:162-8)** 1291

APPROVED FELLOWSHIP TRAINING PROGRAMS

American Board of Obstetrics and Gynecology approved fellowship training programs in obstetrics and gynecology subspecialties: Gynecologic Oncology; Reproductive Endocrinology; Maternal-Fetal Medicine 30A

Information for authors on pages 27A, 28A, and 29A

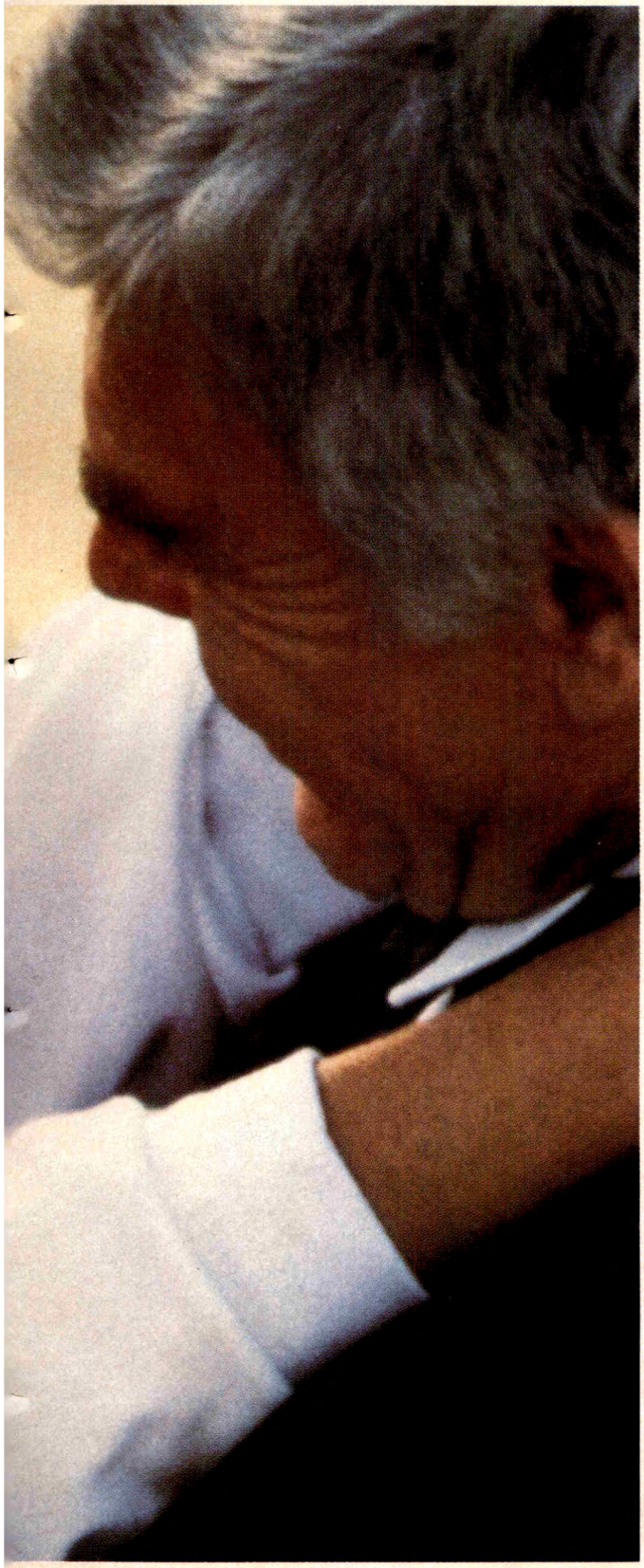
Index to advertisers on page 68A

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Vaginal Cream

0.625 mg/g



BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.
Three independent, case-controlled studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case-controlled studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semi-annual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equi-estrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing, in later life, a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30% to 90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case-controlled study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: PREMARIN (conjugated estrogens tablets, USP): Moderate-to-severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms and they should not be used to treat such conditions.) Osteoporosis (abnormally low bone mass). Atrophic vaginitis. Kraurosis vulvae. Female castration.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Concomitant Progestin Use: The lowest effective dose appropriate for the specific indication should be utilized. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of the endometrium suggest that 10 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks which may be associated with the inclusion of progestin in estrogen replacement regimens. (See PRECAUTIONS.) The choice of progestin and dosage may be important; product labeling should be reviewed to minimize possible adverse effects.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (see Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Estrogens have been reported to increase the risk of endometrial carcinoma (see Boxed Warning). However, a recent large, case-controlled study indicated no increase in risk of breast cancer in postmenopausal women. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not yet complete. If concomitant progestin therapy is used, potential risks may include adverse effects on carbohydrate and lipid metabolism.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. However, in a recent, large case-controlled study of postmenopausal women there was no increase in risk of breast cancer with use of conjugated estrogens.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.

2. *Given cyclically:* Osteoporosis. Female castration. Osteoporosis —0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off). Female castration—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (eg, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

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1. Lindsay R, Hart DM, Clark DM: The minimum effective dose of estrogen for prevention of postmenopausal bone loss. *Obstet Gynecol* 1984;63:759-763. **2.** Studd JWW, Thom MH, Paterson MEL, et al: The prevention and treatment of endometrial pathology in postmenopausal women receiving exogenous estrogens, in Pasetto N, Paoletti R, Ambrus JL (eds): *The Menopause and Postmenopause*. Lancaster, England, MTP Press Ltd, 1980, chap 13.

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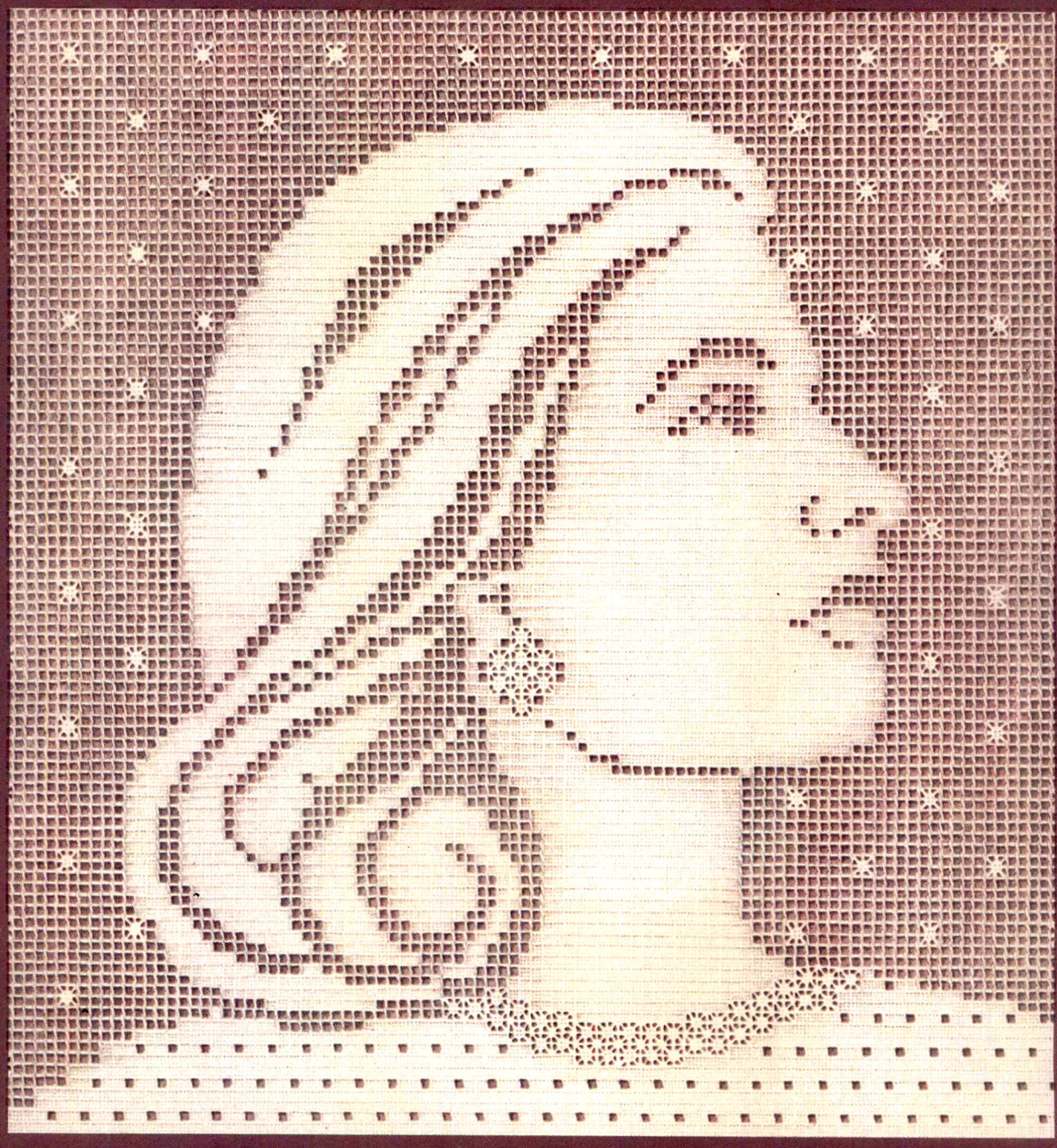
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LEVLEN™

(Levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg tablets)

The lowest total hormonal dose in a monophasic OC formula

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. The physician should be aware of the complete prescribing information for this product. Please also see brief summary of prescribing information on the next page.

BERLEX™ Dedicated to innovation in OB/GYN medicine

LEVLEN™ 28
LEVLEN™ 21

(Levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg tablets)

BRIEF SUMMARY

Each Levlen tablet contains 0.15 mg of levonorgestrel (d (-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.03 mg of ethinyl estradiol (19-nor-17 α-pregna-1,3,5 (10)-trien-20-yne-3,17-diol) (7 pink inert tablets are included in Levlen 28).

Indications and Usage—Levlen is indicated for the prevention of pregnancy in women who elect to use OCs as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only OCs have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. (The rates given for Pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only OCs are used), and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving OCs, since the drugs are more effective in preventing intrauterine than ectopic pregnancies.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination being performed.

2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See patient package labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs:
a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thryoid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis, Mutagenesis, Impairment of Fertility—See "Warnings" section #3, 4 and 5 for information on carcinogenesis, mutagenesis and impairment of fertility.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings." Because of the potential for adverse reactions in nursing infants from OC tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis; pulmonary embolism; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; benign hepatomas; congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria; hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Levlen must be taken exactly as directed and at intervals not exceeding 24 hours.

Any time the patient misses 1 or 2 light-orange tablets, she should also use another method of contraception until she has taken a light-orange tablet daily for 7 consecutive days.

For full details on dosage and administration, see prescribing information in package insert.



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American Journal of Obstetrics and Gynecology

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INFORMATION FOR AUTHORS

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The requirements for manuscripts submitted to the *American Journal of Obstetrics and Gynecology* conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C. V. Mosby Company or the Editors on request.

Manuscripts should be submitted to one of the three Editors as follows: (1) Dr. Brewer—all articles originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians; (2) Dr. Zuspan—articles from the northeastern quadrant of the United States, Japan, Israel, Italy, and England, articles written for Clinical Opinion and Current Development, and Letters to the Editors; (3) Dr. Quilligan—articles from the north central states (including Ohio), states west of the Mississippi, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for articles from the Society for Gynecologic Investigation. Manuscripts should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033. Dr. Zuspan is responsible for articles from The Society of Perinatal Obstetricians. Manuscripts should be submitted to Dr. John A. Read, 9102 Lake Steilacoom Point Road, S.W., Tacoma, WA 98498.

Most of the provisions of the Copyright Act of 1976 became effective on January 1, 1978. **Therefore, all manuscripts must be accompanied by the following written statement, signed by one author:** "The undersigned author transfers all copyright ownership of the manuscript [title of article] to The C. V. Mosby Company in the event the work is published. The undersigned author warrants that the article is original, is not under consideration by another journal, and has not been previously published. I sign for and accept responsibility for releasing this material on behalf of any and all co-authors." Authors will be consulted, when possible, regarding republication of their material.

When submitting a manuscript, an author should include, if applicable, a full statement about all submissions and prior reports in any medium that might be regarded as prior or duplicate publication of the same or similar work. Copies of such material should be included with the submitted manuscript. Articles previously published in another language will not be considered.

It is assumed by the Editors that articles emanating from a particular institution are submitted with the approval of the requisite authority. Articles dealing with human experimentation that require local institutional approval must have this approval prior to submission of the article for publication and it should be so indicated in the Methods section. Reports of experiments on animals must indicate which guidelines for the care and use of the animals were followed.

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tor(s) and Publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the Publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality copies of the manuscript and three glossy prints of illustrations are required.

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

Title page. The title page should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of all authors and their highest academic degree (both M.D. and Ph.D. are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; acknowledgement of source(s) of support; presented line, if applicable; disclaimers, if any; name and address of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name and address of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Abstract page and key words/phrases. On manuscript page two is the abstract, also typed double spaced with the required margins and headed by the title of the article and author(s) name(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations since the emphasis is on the "action" rather than the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods section* describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results section* present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your observations to those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment section* state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number, except for case reports and brief communications, limited to 2, and Current Development, for which there is no limit. Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals." Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Illustrations and tables. A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for color reproduction. For color photographs, submit original transparencies and two sets of unmounted prints on glossy paper with good color balance. For black and white illustrations, submit three sets of 3 by 4 inch (minimum) to 5 by 7 inch (maximum) unmounted glossy photographic prints. Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with author(s) name(s), title of article, number, and top marked clearly). Do not use paper clips. Legends must accompany all figures; type double spaced on a single sheet of paper if possible and number with manuscript in sequence after the references. Titles should be included in the legend, *not* on the print.

All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black and white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted. Illustrations will be returned only on the author's request.

Tables should be typed on separate sheets of paper and

Estimating length of manuscript

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200 word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7 page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double spaced with the required 1 inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 typed words in manuscript. Thus if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

included at the end of the text, one to a page. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table and each column precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

Permissions and photographic consent. Previously published tables and figures must be correctly referenced and written permission from the copyright holder included with the manuscript. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for a regular article.

Clinical Opinion. Limit of 3000 words, 16 references. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double spaced. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

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Fellowships

American Board of Obstetrics and Gynecology approved fellowship training programs in obstetrics and gynecology subspecialties: Gynecologic Oncology; Reproductive Endocrinology; Maternal-Fetal Medicine

CATEGORY State: City Institution	Number of approved positions	Director of program
GYNECOLOGIC ONCOLOGY		
Alabama: Birmingham		
Univ Ala	1 per year (total 2)	Kenneth D. Hatch
California: Los Angeles		
LAC/USC Med Ctr	1 per year (total 2)	C. Paul Morrow
UCLA School Med	1 per year, alt 2 per year (total 3)	Jonathan S. Berek
California: Orange		
Univ Calif Irvine Med Ctr	1 per year, alt 2 per year (total 3)	Philip J. DiSaia
Connecticut: New Haven		
Yale Univ	1 per year (total 2)	Peter E. Schwartz
DC: Washington		
Georgetown Univ Med Ctr	1 per year (total 2)	Gregorio Delgado
Walter Reed Army Med Ctr	1 per year (total 2)	Robert C. Park
Florida: Miami		
Univ Miami/Jackson Memorial	1 per year, alt 2 per year (total 3)	Hervy E. Averette
Florida: Tampa		
Univ So Fla	1 per year (total 2)	Denis Cavanagh
Illinois: Maywood		
Loyola Univ Med Ctr	1 per year (total 2)	John H. Isaacs
Kentucky: Lexington		
Univ Ky Med Ctr	1 per year (total 2)	John R. van Nagell, Jr.
Maryland: Baltimore		
Johns Hopkins Hosp	1 per year (total 2)	John L. Currie
Massachusetts: Boston		
Brigham & Women's Hosp	1 per year (total 2)	Robert C. Knapp
Mass Gen Hosp	1 per year (total 2)	Arlan F. Fuller
Michigan: Ann Arbor		
Univ Mich Med Ctr	1 per year (total 2)	James A. Roberts
Minnesota: Minneapolis		
Univ Minn	1 per year (total 2)	Leo B. Twiggs
Minnesota: Rochester		
The Mayo Clinic	1 per year (total 3) (3 year program)	Karl C. Podratz
Missouri: St. Louis		
Washington U/Barnes Hosp	1 alt years (total 1)	Ming-Shian Kao
New York: Brooklyn		
SUNY Health Sci Ctr/Brooklyn	1 alt years (total 1)	John G. Boyce
New York: Buffalo		
Roswell Park Memorial Inst	1 per year (total 2)	M. Steven Piver
New York: New York		
Memorial Sloan-Kettering	2 per year (total 4)	John L. Lewis, Jr.
Mt. Sinai Med Ctr	1 alt years (total 1)	Carmel J. Cohen
New York: Rochester		
Univ Rochester	1 per year (total 2)	Jackson B. Beecham

Fellowships are listed aphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
GYNECOLOGIC ONCOLOGY—Cont'd		
North Carolina: Chapel Hill		
Univ NC	1 per year (total 2)	Wesley C. Fowler, Jr.
North Carolina: Durham		
Duke Univ Med Ctr	1 per year (total 2)	Daniel L. Clarke-Pearson
Pennsylvania: Hershey		
Milton S. Hershey Med Ctr	1 alt years (total 1)	Rodrigue Mortel
Pennsylvania: Philadelphia		
Hosp Univ Pa	1 per year (total 2)	John J. Mikuta
Texas: Dallas		
Univ Tex/Southwest Med School	1 per year (total 3) (3 year program)	Roberto Yazigi
Texas: Houston		
M. D. Anderson Hosp	3 per year (total 6)	J. Taylor Wharton
REPRODUCTIVE ENDOCRINOLOGY		
Alabama: Mobile		
Univ So Ala	1 per year (total 2)	Sezer Aksel
California: La Jolla		
Univ Calif San Diego	2 per year (total 4)	Samuel S. C. Yen
California: Los Angeles		
LAC/USC Med Ctr	3 per year (total 6)	Rogerio A. Lobo
UCLA School Med	1 per year, alt 2 per year (total 3)	Howard L. Judd
California: San Francisco		
Univ Calif San Francisco	2 per year (total 4)	Robert B. Jaffe
Connecticut: Farmington		
Univ Conn School Med	1 per year (total 2)	Anthony A. Luciano
Connecticut: New Haven		
Yale Univ School Med	3 per year (total 6)	Alan H. DeCherney
DC: Washington		
George Washington Univ	1 per year (total 2)	Robert J. Stillman
Georgetown Univ	1 per year (total 2)	Richard J. Falk
Walter Reed Army Med Ctr	3 per year (total 6)	Thomas A. Klein
Florida: Miami		
Univ Miami	1 per year (total 2)	William J. LeMaire
Georgia: Augusta		
Med Coll Ga	2 per year (total 4)	Paul G. McDonough
Illinois: Chicago		
Michael Reese Hosp	2 per year (total 4)	Edward L. Marut
Northwest Univ	2 per year (total 4)	Robert W. Rebar
Rush Med Coll	1 per year (total 2)	W. Paul Dmowski
Univ Ill	2 per year (total 4)	M. Yusoff Dawood
Kentucky: Lexington		
Univ Ky	1 per year (total 2)	Emery A. Wilson
Kentucky: Louisville		
Univ Louisville School Med	1 per year (total 2)	Marvin A. Yussman
Lousiana: New Orleans		
La State Univ	1 per year (total 2)	Krishna B. Singh
Tulane School Med	1 per year (total 2)	Ian H. Thorneycroft
Maryland: Baltimore		
Johns Hopkins Univ	2 per year (total 4)	John A. Rock
Univ Md	1 per year (total 2)	Eli Y. Adashi

Fellowships are listed alphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
REPRODUCTIVE ENDOCRINOLOGY—Cont'd		
Massachusetts: Boston		
Beth Israel Hosp	1 per year (total 2)	Machelle M. Seibel
Brigham & Women's Hosp	3 per year (total 6)	Isaac Schiff
Michigan: Detroit		
Wayne State Univ/Hutzel Hosp	1 per year, alt 2 per year (total 3)	Kamran S. Moghissi
Minnesota: Minneapolis		
Univ Minn	1 per year (total 2)	George E. Tagatz
Minnesota: Rochester		
The Mayo Clinic	1 per year (total 2)	Steven J. Ory
Missouri: St. Louis		
Washington Univ	1 per year (total 2)	James C. Warren
New Jersey: New Brunswick		
UMDNJ/Robt. W. Johnson Med School	1 per year (total 2)	Ekkehard Kemmann
New Jersey: Newark		
UMDNJ/New Jersey Med School	2 per year (total 4)	Cecilia L. Schmidt
New York: Brooklyn		
SUNY Health Sci Ctr/Brooklyn	1 per year (total 2)	Francisco I. Reyes
New York: New York		
Columbia Univ	1 per year (total 2)	Raphael Jewelewicz
Mt. Sinai Med Ctr	1 per year (total 2)	J. Victor Reyniak
North Carolina: Chapel Hill		
Univ NC	1 per year (total 2)	Mary G. Hammond
North Carolina: Durham		
Duke Univ Med Ctr	1 per year, alt 2 per year (total 3)	Arthur F. Haney
Ohio: Cleveland		
Case Western/MacDonald Hosp	1 per year (total 2)	Paul T. Schnatz
Ohio: Columbus		
Ohio State Univ	1 per year, alt 2 per year (total 3)	Moon H. Kim
Oregon: Portland		
Oregon Health Sci Univ	1 per year (total 2)	Kenneth A. Burry
Pennsylvania: Philadelphia		
Hosp Univ Pa	2 per year (total 4)	Luigi Mastroianni
South Carolina: Charleston		
Med Univ SC	1 per year (total 2)	H. Oliver Williamson
Tennessee: Memphis		
Univ Tenn	1 per year (total 2)	John E. Buster
Tennessee: Nashville		
Vanderbilt Univ School Med	2 per year (total 4)	Anne C. Wentz
Texas: Dallas		
Univ Tex/Southwest Med School	2 per year, alt 3 per year (total 5)	Bruce R. Carr
Texas: Houston		
Baylor Coll Med	2 per year (total 4)	William E. Gibbons
Univ Tex/Houston	1 per year (total 2)	Craig A. Winkel
Texas: San Antonio		
Univ Tex/San Antonio	1 per year (total 2)	Robert S. Schenken
Vermont: Burlington		
Univ Vt	1 per year (total 2)	Mark Gibson
Virginia: Charlottesville		
Univ Va School Med	1 alt years (total 1)	James D. Kitchin III
Virginia: Norfolk		
East Va Med School	2 per year (total 4)	Zev Rosenwaks

Fellowships are listed aphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
REPRODUCTIVE ENDOCRINOLOGY—Cont'd		
Wisconsin: Madison		
Univ Wis	1 per year (total 2)	Sander S. Shapiro
Canada: London (Ont)		
Univ West Ont	1 alt years (total 1)	Robert F. Casper
Canada: Montreal (Que)		
McGill Univ	1 per year (total 2)	A. Brian Little
MATERNAL-FETAL MEDICINE		
Alabama: Birmingham		
Univ Ala	1 per year, alt 2 per year (total 3)	John C. Hauth
Alabama: Mobile		
Univ So Ala	1 per year (total 2)	Micki L. Cabaniss
Arizona: Tucson		
Univ Ariz Health Sci Ctr	1 per year (total 2)	Louis Weinstein
Arkansas: Little Rock		
Univ Ark	1 per year (total 2)	J. Gerald Quirk, Jr.
California: La Jolla		
Univ Calif San Diego School Med	1 per year (total 2)	Robert Resnik
California: Loma Linda		
Loma Linda Univ	1 per year (total 3) (3 year program)	Larry M. Cousins
California: Long Beach		
Univ Calif Irvine Med Ctr	3 per year (total 6)	Thomas J. Garite
California: Los Angeles		
Harbor/Cedars-Sinai Med Ctrs	3 per year (total 6)	Calvin J. Hobel
King-Drew Med Ctr	1 per year (total 2)	Ezra C. Davidson, Jr.
LAC/USC Med Ctr	4 per year (total 8)	Richard H. Paul
Univ Calif Los Angeles Med Ctr	1 per year (total 2)	Charles R. Brinkman III
California: San Francisco		
Univ Calif San Francisco	4 per year (total 8)	Julian T. Parer
California: Stanford		
Stanford Univ	1 per year (total 2)	Kent Ueland
Colorado: Denver		
Denver Gen Hosp	1 alt years (total 1)	John G. McFee
Univ Colo School Med	1 per year (total 2)	Edgar L. Makowski
Connecticut: Farmington		
Univ Conn Health Ctr	2 per year (total 4)	David J. Nochimson
Connecticut: New Haven		
Yale Univ	1 per year (total 2)	John C. Hobbins
DC: Washington		
George Washington Univ	1 per year, alt 2 per year (total 3)	John H. Grossman III
Georgetown Univ School Med	1 per year (total 2)	John T. Queenan
Florida: Gainesville		
Univ Fla/Shands Hosp	1 per year (total 2)	Amelia C. Cruz
Florida: Miami		
Univ Miami/Jackson Memorial	2 per year (total 4)	Mary Jo O'Sullivan
Florida: Tampa		
Univ So Fla/Tampa Gen	2 per year (total 4)	Robert A. Knuppel
Georgia: Augusta		
Med Coll Ga	1 per year (total 2)	Lawrence D. DeVoe
Illinois: Chicago		
Chicago Lying-In Hosp	1 per year (total 2)	Atef H. Moawad

Fellowships are listed aphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
MATERNAL-FETAL MEDICINE—Cont'd		
Northwest Univ	2 per year, alt 3 per year (total 5)	Michael L. Socol
Rush Presbyterian/St. Lukes	1 per year (total 2)	Howard T. Strassner, Jr.
Univ Ill Coll Med	2 per year (total 4)	Stanley A. Gall
Illinois: Springfield		
South Ill Univ	1 per year (total 2)	Kofi S. Amankwah
Indiana: Indianapolis		
Indiana Univ School Med	1 per year (total 2)	Alan M. Golichowski
Louisiana: New Orleans		
State Univ/Charity	1 per year (total 2)	Harvey A. Gabert
Maryland: Baltimore		
Johns Hopkins Hosp	1 per year, alt 2 per year (total 3)	Jennifer R. Niebyl
Univ Md	1 per year (total 2)	David A. Nagey
Massachusetts: Boston		
Brigham & Women's Hosp	2 per year (total 4)	Fredric D. Frigoletto
Tufts Univ/St. Margaret's	1 per year (total 2)	Curtis L. Cetrulo
Michigan: Detroit		
Wayne State Univ/Hutzel Hosp	2 per year (total 4)	Frederico G. Mariona
Mississippi: Jackson		
Univ Miss Med Ctr	2 per year (total 4)	John C. Morrison
Missouri: St. Louis		
Washington Univ Med Ctr	2 per year (total 4)	Roy H. Petrie
New Jersey: New Brunswick		
UMDNJ/Robt. W. Johnson Med School	1 per year (total 2)	John T. Harrigan
New Jersey: Newark		
UMDNJ/St. Joseph's Hosp	1 per year (total 2)	James P. Thompson
New Mexico: Albuquerque		
Univ NM	1 per year (total 2)	Gloria E. Sarto
New York: Brooklyn		
SUNY Health Sci Ctr/Brooklyn	1 per year (total 2)	Howard Minkoff
New York: Bronx		
Albert Einstein Coll Med	2 per year (total 4)	Irwin R. Merkatz
New York: Buffalo		
SUNY/Children's Hosp	1 per year (total 2)	William P. Dillon
New York: East Meadow		
Nassau County Med Ctr	1 per year (total 2)	Nergesh A. Tejani
New York: Mineola		
Winthrop-Univ Hosp	1 per year (total 2)	Harold Schulman
New York: New York		
Columbia Univ	1 per year (total 2)	Harold E. Fox
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NY Univ Med Ctr	1 per year (total 2)	Bruce K. Young
New York: Rochester		
Univ Rochester/Strong Memorial	1 per year (total 2)	James R. Woods, Jr.
New York: Syracuse		
SUNY Health Sci Ctr/Syracuse	1 per year (total 2)	Shiraz Sunderji
North Carolina: Chapel Hill		
Univ NC	1 per year (total 2)	John W. Seeds
North Carolina: Durham		
Duke Univ Med Ctr	1 per year (total 2)	Allen P. Killam
North Carolina: Winston-Salem		

Fellowships are listed aphabetically by state.

CATEGORY State: City Institution	Number of approved positions	Director of program
MATERNAL-FETAL MEDICINE—Cont'd		
Bowman Gray School Med	1 per year (total 2)	Paul J. Meis
Ohio: Cincinnati		
Univ Cincinnati Med Ctr	2 per year (total 4)	Thomas P. Barden
Ohio: Cleveland		
Case Western/MacDonald Hosp	1 per year (total 2)	John P. O'Grady
Cleveland Metro Gen Hosp	2 per year (total 4)	LeRoy J. Dierker, Jr.
Ohio: Columbus		
Ohio State Univ	1 per year (total 2)	Richard O'Shaugnessy
Oklahoma: Oklahoma City		
Univ Ok/Coll Med	1 per year (total 2)	Warren M. Crosby
Oklahoma: Tulsa		
Univ Ok/Tulsa Med Coll	1 per year (total 2)	Larry J. D'Angelo
Oregon: Portland		
Oregon Health Sci Univ	1 per year (total 2)	John M. Bissonnette
Pennsylvania: Philadelphia		
Hosp Univ Pa	1 per year (total 2)	Michael T. Mennuti
Pa Hosp	2 per year (total 4)	Ronald J. Bolognese
Temple Univ/Albert Einstein	1 alt years (total 1)	Arnold W. Cohen
Thomas Jefferson Univ	1 per year (total 2)	Ronald J. Wapner
Pennsylvania: Pittsburgh		
Univ Pittsburgh/Magee-Women's	2 per year (total 4)	Eberhard Mueller-Heubach
Rhode Island: Providence		
Brown Univ/Women's & Infant's	1 per year (total 2)	Donald R. Coustan
South Carolina: Charleston		
Med Univ SC	1 per year (total 2)	Edgar O. Horger III
Tennessee: Memphis		
Univ Tenn	2 per year (total 4)	Baha M. Sibai
Tennessee: Nashville		
Vanderbilt Univ Med Ctr	1 per year (total 2)	Frank H. Boehm
Texas: Dallas		
Univ Tex/Southwest Med School	3 per year, alt 4 per year (total 7)	Larry C. Gilstrap III
Texas: Houston		
Baylor Coll Med	2 per year (total 4)	Clark M. Hinkley
Univ Tex Med School/Houston	2 per year (total 4)	Valerie M. Parisi
Texas: San Antonio		
Univ Tex/San Antonio	2 per year (total 4)	Ronald S. Gibbs
Utah: Salt Lake City		
Univ Utah	1 per year (total 3) (3 year program)	Neil K. Kochenour
Vermont: Burlington		
Univ Vt	1 per year (total 2)	Eleanor L. Capeless
Virginia: Norfolk		
East Va Med School	1 per year (total 2)	Donald L. Levy
Washington: Seattle		
Univ Wash	1 per year (total 2)	Thomas J. Benedetti
Washington: Tacoma		
Madigan Army Med Ctr	1 per year (total 2)	John A. Read
Wisconsin: Madison		
Univ Wis Med Ctr	2 per year (total 4)	Luis B. Curet

Fellowships are listed aphabetically by state.

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URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Pseudomonas* sp. including *Ps. aeruginosa; Proteus mirabilis; P. vulgaris; Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp.; *Serratia* sp. including *S. marcescens; Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae.*

INTRA-ABDOMINAL INFECTIONS caused by *Escherichia coli; Staphylococcus epidermidis; Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Bacteroides* sp. including *B. fragilis; Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis; Escherichia coli; Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis; Serratia* sp.; *Enterobacter* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis; Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae.* 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae.*

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

***Pseudomembranous colitis* has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who developed diarrhea in association with antibiotic use.** Treatment with broad-spectrum antibiotics alters normal flora of the colon and may permit overgrowth of *Clostridia.* Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro.* Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile.* Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Careful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox,' nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established.

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should be exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not been established. In children six months of age and older, treatment with 'Cefizox' has been associated with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatine phosphokinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the *most* frequent adverse reactions (*greater than* 1%, but less than 5%) are: *Hypersensitivity*—Rash, pruritus, fever. *Hepatic*—Transient elevation in SGOT, SGPT and alkaline phosphatase. *Hematologic*—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. *Local*—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (*less than* 1%) are: *Hypersensitivity*—Numbness has been reported rarely. *Hepatic*—Elevation of bilirubin has been reported rarely. *Renal*—Transient elevations of BUN and creatinine have been occasionally observed with ceftizoxime. *Hematologic*—Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. *Genitourinary*—Vaginitis has rarely occurred. *Gastrointestinal*—Diarrhea; nausea and vomiting have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams of ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxime.

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams of ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufactured for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

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References: 1. Lou, M.A., et al.: Comparative evaluation of ceftizoxime and cefoxitin in the treatment of intraabdominal infections, 14th Internat. Congr. Chemother., Kyoto, Japan, June 1985, pp. 2378-2379. 2. Fu, K.P., and Neu, H.C., Antibacterial activity of ceftizoxime, a ß-lactamase-stable cephalosporin, *Antimicrob. Agents Chemother.* 17:583-590, 1980. 3. Thornsberry, C.: Review of in vitro activity of third-generation cephalosporins and other newer beta-lactam antibiotics against clinically important bacteria, *Am. J. Med.* 79(Suppl. 2A): 14-20 (Aug.) 1985.

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CLINICAL SECTION

Clinical Opinion

Stress in residency: Proposals for solution

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Ann Arbor, Michigan, and Denver, Colorado

Current problems leading to excessive stress in residency are presented and are compared with those of a generation ago. Solutions are proposed that can be summarized as follows: (1) commitment by the program director and chairman to resolving stress in residency and (2) flexibility by the American Board of Obstetrics and Gynecology and the Residency Review Committee. A general discussion is presented. (AM J OBSTET GYNECOL 1987;157:1093-6.)

Key words: Stress, residency, substance abuse

Although an increasing number of papers in recent years have discussed stress during residency training, little has been specifically written about residents in obstetrics and gynecology. A survey of residency training programs in six medical specialties indicated that obstetrics and gynecology residencies rank low in offers of support that would help them cope with the stress of postgraduate training.[1] Most residency directors and department chair persons are aware of depression, substance abuse, and suicide attempts in their residency groups. It is time for the specialty of obstetrics and gynecology to examine this problem from the general perspective of factors that affect residents in any specialty and from the unique perspective of obstetrics and gynecology. For years the stresses of residency training have included identity crisis, sleep deprivation, financial problems, and marital problems; however, we believe that new factors make these stresses more acute and will be discussed in this article.

General residency stresses

Finances. Financial problems have become a major stress, with the average medical student incurring a debt of $32,000, even commonly as high as $40,000.[2] This is a staggering figure, which presents an additional stress today because traditional sources for subsidizing such debts are unavailable. Young physicians find banks much less willing to loan than they use to, perhaps because they feel that physicians manage their money poorly or more likely because the future financial strength of a physician is not as bright as in the past. Even though resident salaries have increased substantially in the past 20 years, it has not been enough to make a significant difference. In 1966, a postgraduate year IV resident earned $4,000, which translates into $19,000 today. In contrast, a PGY IV resident today earns $27,000 but has a much larger debt service to manage. Salaries and benefits may be negotiated by a union (House Officer Association), whose management has little relationship with educational realities.

Other indirect sources of financial support in the past have also disappeared. In large communities, the hospitals often owned or managed an apartment building, which allowed most of the house staff and their families to live in the same facility so that there was a support network for the residents and their families. These facilities were usually offered inexpensively and helped subsidize the lower salaries. Today's residents are scattered throughout their communities in less friendly rentals or expensive mortgaged homes. The support is lacking and problems are compounded.

Moonlighting was uncommon in the past because few jobs were available and it was frequently prohibited.

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Jobs such as insurance physicals, at $5 to $15 apiece, were not a very productive way to earn money. Emergency room jobs were rare at that time. Today the house officer has a large number of sites at which to moonlight, providing some income but interfering greatly with family well-being and adding the stress of professional liability.

Finally, residents must be affected by the predictions for a gloomy economic future in the United States. Inflation makes retirement and health care plans unreliable. Recession or depression can destroy any plans. Income is predicted to flatten or fall.

Substance abuse. The American Medical Association[3] estimates that at least 10% of physicians are now or will be alcoholics or drug addicts. A survey of residents at Columbia Presbyterian Medical Center in New York showed that 12.7% were likely to have an alcohol problem.[4] The work of McAuliffe et al.[5,6] demonstrates the increasing use of psychoactive drugs among younger physicians since recreational drug use became more popular in the 1960s. In their studies, more than half of young physicians had used psychoactive drugs, including recreational use of marijuana and cocaine and self-treatment with tranquilizers or opiates. They found that 10% of physicians reported current regular drug use (once a month or more often) and that 3% were drug dependent.[5] Alcohol has been the traditional recreational substance of choice during the training years and has led some to drug abuse and alcoholism. Clearly, a new factor is the increasing social acceptance of recreational drug use and the inherent risks for developing drug abuse problems and addiction, especially with the availability of samples of addictive drugs such as minor tranquilizers.

Marital discord. Residency is a time of intense personal transition with the added stress of long work hours that take residents away from their families. Marital tension may result and is an additional stress that may contribute to impairment of performance. As more women enter our residency programs, it is important to realize that the divorce rate for female physicians is higher than for male physicians.

Since 80% of female physicians marry other professionals, dual-career issues have become increasingly prominent and present the problems of finding positions in the same area for both partners, difficulties in planning leisure time together, and difficulties in arranging for pregnancy and child care. These stresses are even worse if the professional is also a resident.[7]

Sleep deprivation. Call schedules today are supposedly easier than in the past and usually occur every third or fourth night; however, patient care is now much more stressful with more opportunities for mistake. The increasing complexity of medical care with a continuing trend toward subspecialization has made decision making while on call difficult and frustrating since the resident may feel unprepared to face these decisions alone. Residents are acutely aware of the increasing number of malpractice suits and skyrocketing malpractice insurance premiums in obstetrics and gynecology, and their worries about these add another burden to the decision-making process while alone on call. These factors lead to increased on call stress and the consequences of such stress. Although overwork and fatigue are not thought to be causes in and of themselves for substance abuse, depression, and suicide, they are increasingly considered contributing factors that may be the straw that breaks the camel's back.[8] Recent evidence indicates that being on call significantly increases pulse rate and blood pressure.[9]

Identity. Residency training is the period when important developmental tasks of early adult life are consolidated, including work, play, mentor relationships, attitudes toward care of one's body, and concerns about time limitations and death.[10] This developmental process is greatly affected by the areas of stress that have already been discussed. In addition, the future of our house officers is much less certain than it previously was. In the past we could hang out a shingle in a place where we wanted to live and were virtually guaranteed of success. This is no longer true, and many of our house officers have trouble finding a practice position. In fact, the practice of opening a practice has now changed into looking for a job. The atmosphere is different, the opportunities are less, and the future is not as bright.

The professional world in which we live is certainly less happy than it was in the past. Patients are much more argumentative and in some cases threatening. The charge of sexual abuse can occur at any time. The spectre of professional liability, whether frivolous or not, is great. Risk management and morbidity and mortality discussions involve house staff much more frequently than they did in the past. The horror of contracting diseases such as sexually transmitted disease, herpes, or acquired immune deficiency syndrome from patient care can be overwhelming. Advocate groups for the poor, the handicapped, and others can be disruptive and even threatening.

Subspecialization is rapidly occurring in all specialties with an increasing emphasis on board certification and subspecialty boards. Subspecialty education can disrupt residencies and the decision whether to subspecialize adds stress. Board examinations are more comprehensive and the failure rate for the American Board of Obstetrics and Gynecology is still 20%.

Mentor relationships are still important, but as medical life becomes increasingly complicated, these relationships are more difficult to form. Faculty and chair persons today move more frequently, which again in-

terferes with the resident's ability to form faculty relationships.

Overall, the resident's developmental needs are impaired by the factors discussed. This leads to a much less positive attitude about the future and makes it difficult for residents to cope with the stresses they encounter.

Solutions

There are no easy solutions to this problem; however, change can be achieved if the necessary steps are taken in a careful and planned fashion, which will result in a slow evolutionary process in medical training in the United States. Important barriers may hinder programs from adequately addressing the problems of resident stress and impairment.[11] These include a lack of focus on primary prevention, problems in the supervisory process that place a priority on teaching and not on issues of residency stress, resident resistance to admitting they are under stress and are in need of help, and stigmatization and rejection of residents by faculty and other residents when stress is discussed. Clearly, any major change in residency training will involve a drastic change in attitudes and priorities about what is important in residency training. Attitudes such as these will take time to modify. The important point is that the time to start is now.

We propose the following solutions to the problem of residency stress:

1. There must be a total commitment to change the residency program by the program director and faculty. This commitment would be directly observable if the residency director and chair person were the same individual. If not, the residency director and chair person must work so closely that the authority of the chair is visibly vested in the residency director. With this commitment and leadership, faculty and residents will know reduction in residency stress is a priority.
2. Mentors are a recently rediscovered phenomenon in medical education. It would be helpful in residency training to use this model and assign house officers to the chair person or residency director for the first year with the requirement they must select a different faculty member as mentor at the end of that year. This allows the house officer to get to know various faculty members during the first year and choose someone whom they respect and with whom they have a rapport to work with throughout the remainder of the residency. The mentor relationship can provide support, role modeling, and advice about clinical, professional, and personal issues.
3. Faculty and senior residents must vigorously organize support groups. Even though residents live throughout the community, it is important that they are able to provide mutual support in facing professional stresses, illnesses, and family issues such as pregnancy, child care, and recreational time.
4. House officers must be involved in decisions concerning their residency. Many times they are more aware of problems than are the faculty and their ideas are valuable A committee concerned with the well-being of the house staff should be formed to give resident representatives a formal forum in which to present problems and ideas for solutions to the residency director and faculty representatives. Such committees must have power for the residents to feel that they are able to work effectively with their problems.[12] Within general guidelines set by the program director and the certifying agencies, residents (chief administrative resident) should arrange call and vacation schedules. Family time can be enhanced, and coordination with spouse call or work schedules can be arranged.
5. Institutions should provide counseling on a readily available basis, and counseling must be private and confidential.[13] Confidentiality is enhanced if psychotherapy is available off campus.
6. Recent national publicity is indicative of the crisis we have reached in the area of fatigue and its effect on house officer performance and education. The call schedule system must be changed. If residents are awake most of the night, they must be allowed to go home the next morning to sleep. If they are not awake all night, they should be allowed to go home the next morning to freshen up and return for an afternoon of less stressful duties such as research or paperwork.
7. Residents should be involved frequently in social events for the whole department to relax and feel the involvement and support of the faculty.
8. Finally, we need to take a serious look at how residency training is structured. It is mandated by both the American Board of Obstetrics and Gynecology, Inc. and the Residency Review Committee. There must be 4 years of education after medical school; 36 months of this must be in obstetrics and gynecology. There can be no more than 6 weeks' absence during any year of residency or makeup must occur. The resident must see an unwritten minimum number of cases for the residency to be approved. This same model is applied to all programs and all residents.

Programs must be allowed to vary. Those with large numbers of deliveries and/or surgical cases can graduate a resident in 4 years, the minimum time required to master a complex multidisciplinary specialty such as

Obstetrics and Gynecology. Programs with smaller numbers of cases may require 5 or 6 years to graduate a resident. By the same token, residents must be allowed to vary the structure of their training according to their level of skill. The length of training for an individual resident should be flexible without penalty from the Residency Review Committee or American Board of Obstetrics and Gynecology. Currently a resident can be held back only with prior permission by the Residency Review Committee, and two residents cannot be held back simultaneously. These requirements force residency programs to graduate residents on time whether or not they are ready for independent practice. Variable programs may lead to legal problems with claims of discrimination; however, if educational objectives are clearly written, carefully followed, and documented in writing, variations in resident training should be allowable.

Residency stress is a significant problem today and differs substantially from the past. We have suggested solutions for resolution of this problem, but it is clear that the solutions are expensive and will take years to implement. If we are committed to make residency stress a priority, barriers such as financial cost can be overcome.

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The pregnant brain dead and the fetus: Must we always try to wrest life from death?

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This article deals with the ever more timely and often vexing topic of maintaining a brain-dead mother as an incubator for her developing offspring. It explores the issue by: (1) reviewing the history of the problem and the "state of the art" today, (2) examining the moral problem of using brain-dead persons as incubators for potential or actual others, (3) searching for moral differences between maternal death early or late in pregnancy, and (4) presenting a possible resolution in such tragic cases. It concludes that (1) a moral necessity to deliver viable infants from brain-dead mothers exists; (2) the farther from viability brain death occurs, the more maintaining the mother as an incubator resembles experimental therapy with its imperative for careful, informed consent; (3) experimental therapy not being morally necessary, its proceeding under these tragic circumstances should invoke community support for the next of kin in dealing with the immediate and long-term costs; (4) all ethical problems proceed in a context to which the moral actors must be sensitive and one that alters the conclusions made. (Am J Obstet Gynecol 1987;157:1097-101.)

Key words: Brain death in pregnancy, postmortem cesarean section, physician/patient relationship

The current work explores the issue of artificially maintaining a brain-dead pregnant patient long enough that a potentially viable child might be delivered. This ever more timely topic is explored by (1) reviewing the history of the problem and the "state of the art" today; (2) examining the issue of using a brain-dead patient merely as an incubator for a potential other and the difference, if any, between that and an actual other; (3) searching for a moral difference between maternal death early or late in pregnancy; and (4) presenting a possible resolution to such tragic problems.

Historical considerations and the state of the art

Cesarean section was apparently first used to extract a living child from a dead, rather than a living child from a living, mother. Although cesarean section may have been practiced in early antiquity, the history of the procedure as well as the origin of the name is unknown. The legendary Greek physician Asklepios, fathered by Apollo, was supposedly delivered from his dead mother, Koronis, by postmortem cesarean section. Bacchus likewise is said to have been born in this manner.[1] No mention of the procedure appears in the works of Hippocrates, Galen, or Soranus. In the East, and in the fourth century BCE, Susruta's Sushruta Samhita mentions a cesarean section done either in "a woman whose life is in great danger" or "in a woman who is dead."[2] The Babylonian Talmud states that if the mother dies during childbirth, the dead mother's abdomen should be immediately opened to deliver the child.[3] This was the practice during Hellenistic times and the Roman authorities in antiquity (and, in the seventeenth century, Venetian authorities) required that the attempt to deliver the living from the dead be made.[4] According to Pliny the Elder, Scipius Africanus was delivered by successful postmortem cesarean section in 237 BCE.[5]

The origin of the term cesarean section is unclear. The *Encyclopedia Britannica*, in its latest edition, states that Pliny attributed it to the family of Julii whose cognomen Cesare (from *caedere*, the Latin for to cut) originated from this alleged manner of their birth. The stories that claim that it is the method by which Julius Caesar was delivered from his dead mother in 102 BCE are evidently apocryphal. Caesar's mother is known to have been very much alive 40 years after her son's birth.[6] About 700 BCE Numa Pompilius forbade the burial of a pregnant woman before the child was removed,[3, 7] but it is unclear whether that was to rescue a live child or to bury it separately. This decree first became part of the King's Law (Lex Regia) and later part of the Emperor's Law or Lex Caesare. The procedure may thus have been termed the Emperor's (or cesarean) operation.

At the Council of Cologne in 1280 CE, the Catholic

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church, following Roman law, required postmortem cesarean sections so that the infant could be properly baptized and buried. Failure to do this was an ecclesiastically punishable offense.[7] Sicilian law in 1749 held that a physician who failed to try such delivery in case of death during late pregnancy was guilty of murder and King Charles ultimately sentenced a physician to death for that failure.[8] In 1757 Austrian law made the procedure compulsory when pregnancy had advanced beyond 6 months. Ecclesiastical and secular law coincided and both required that the attempt be made.

Historically, cesarean section was first performed in an attempt to extract either a living child from a dead mother or, at least, to provide separate baptism and burial; later, the operation was done in a dying mother to save an infant and only quite recently to save both mother and child (the first such operation being successfully done in 1500 by Jacob Nufer, a Swiss sow gelder).[7,8] Today a new twist has been added: Keeping an otherwise dead but biologically alive mother alive merely to enable a nonviable infant to reach viability has added a new dimension.

In 1961, Ritter[1] reviewed recent experience with postmortem cesarean section and suggested that the procedure is practical, even though the prognosis is not good, after 28 weeks' gestation. Ten years later, Weber[6] conducted a careful review of the literature to date and again concluded that pregnancy under 28 weeks offers little if any chance for success. How many actual cases of successful postmortem cesarean section have occurred is, of course, unknowable. However, a recent review, based on a statement in a textbook dealing with trauma in pregnancy, states that "fewer than 200 successful instances of post-mortem Caesarean section have been reported in the literature."[7] Dillon et al. added two cases of their own and concluded that at less than 24 weeks' gestation no "extraordinary measures for mother or fetus" seem warranted. After that, life support for the mother is indicated until cesarean section is performed at 28 weeks to save the child. Earlier cesarean section is done only if maternal viability cannot be maintained.

Postmortem cesarean section is quite different from deliberately maintaining maternal viability for the sake of the fetus. Until recently, brain death meant inevitable whole-body death; therefore the question was moot. Nowadays maintaining viability is at least theoretically possible. Usually the brain dead are maintained for the few hours needed to harvest organs. Keeping brain-dead persons alive for longer than this appears to be a formidable undertaking. In general, it is believed that even with full ventilator and other medical support, cardiac arrest will occur.[9] The occasion to test this thesis rarely arises. A recent case, in which a brain-dead patient was successfully maintained for 74 days until a court order could be obtained,[10] as well as an even more recent case in which biologic existence was maintained for 64 days so that an infant could be delivered, casts doubt on such a thesis.[11] A permanently vegetative patient or one with an irreversible condition poses similar problems. In a case that deserves mention,[12] a patient who was 6 weeks pregnant was in an irreversible coma after an automobile accident. She was delivered of a living normal infant in the thirty-fourth week of gestation. However, maintaining such existence is an incredibly difficult, time-consuming, and costly venture whose success, while possible, is unlikely.

More recent experience has resulted in questioning of the recommendations that state that at less than 24 weeks' gestation no further efforts to sustain the mother are ever justified. Recently a patient who was 22½ weeks pregnant suffered cardiac arrest secondary to a central nervous system lesion; she was resuscitated but was found to be brain dead. Despite bacteremia, diabetes insipidus, adrenal failure, hypothermia, and hypotension she was maintained on a regimen of hyperalimentation until the successful delivery of a normal baby whose eventual chance for survival was deemed to be excellent.[11] Sporadic cases of this sort are being reported from across the country. It is unknown how many attempts fail to produce viable infants or how many result in stunted or defective offspring.

The brain dead as incubators

When brain-dead mothers are maintained for the sake of possibly producing viable offspring, their bodies are used as incubators. No longer are they, in any sense, used as ends in themselves. Every effort to maintain life and combat disease is done not to benefit the person treated but to benefit another. Such persons have become mere means to another's survival.

The situation then has some similarity to the maintenance of the brain dead for harvesting of organs. However, here the newly dead are maintained only briefly, the organs are quickly removed, and elaborate efforts are rarely needed. When it comes to maintaining a brain-dead mother as an incubator for her developing offspring, the situation is closer to maintaining the brain dead indefinitely to grow tissues, do experiments, or otherwise use them as objects.[13] That is sufficiently problematic to bear closer scrutiny.

The physician/patient relationship, as well as respect for rational beings, would seem to prevent physicians from using patients merely as objects to the ends of others. That would preclude not only maintaining brain-dead mothers as incubators for their potential offspring but also maintaining the brain dead, for whatever time, as organ donors; and yet we intuitively feel that there is a clear distinction between the living and the brain dead that changes the nature of obligation.

The physician/patient relationship is one in which both parties, actually or at least potentially, participate.

In medical practice, this relationship is the central issue surrounded and embodied in a complex skein of other relationships in which one or the other party shares. Relationships with patients are dynamic, growing things. When one of the participants dies, growth stops and dynamics cease. The relationship is transmuted. The central core changes and symbolism takes its place; peripheral considerations, those of family, friends, and staff and the needs of others, become the central reality. The newly dead are of great symbolic value as symbols of former persons, but they are symbols and not reality. Their moral value is secondary rather than primary; they are valued not as entities, which have worth in themselves because they are capable of actual or potential harm or benefit (primary value), but because of their symbolic worth and because of their value for others (secondary value). To conflate symbol with reality, to value the symbol more, is to confuse sentiment with sentimentality.

The growing fetus has real as well as symbolic value. Being, at least potentially, capable of being benefited or harmed, it has primary value; being of value (or disvalue) to another, it has secondary value; standing for renewal, continuity, and the life that may yet arise out of the dead, it has symbolic value. Its interests therefore are greater than the interests of its brain-dead mother whose interests can only be looked at symbolically. The interests that before either favored the mother or, in another view, were equivalent now clearly support the fetus.

The growing fetus has real value as a fetus, secondary value (or disvalue) to another, and symbolic value as a symbol of future sentient life. In relation to the brain-dead mother, it is the "flesh of her flesh" and the "blood of her blood," a matter with different standing from using the brain dead as incubators for body parts or as objects for experimentation. It can be viewed as the biologic continuity of her life and the symbolic continuity of her being. These differences may justify the prolonged use of the newly dead for lengthy periods, in fact, for periods that make them no longer "newly" dead.

When the newly dead are to be used to procure organs for the sake of others, we require either previous informed consent, or at least a lack of dissent, of the newly dead or of the next of kin. We require such consent or allow such dissent out of respect for the symbolism inherent in the newly dead. This is done despite the critical need of an actual other whose very life depends on the donation. Whether this is or is not morally the proper thing to do has been widely discussed in the literature.

Maintaining brain-dead patients briefly for the sake of harvesting their organs to be used by living patients who otherwise would be destined to death or severe disability, differs from maintaining brain-dead patients for a substantial length of time to benefit a potential other. Briefly sustaining biologic function to harvest organs expresses respect for the living and affirms our belief that the newly dead were members of a just community concerned with the welfare of its living members. It is a way of valuing the living more than the dead, reality more than its symbol. Doing that (and allowing individual dissent) honors the memory of the dead, supports their symbolic ongoing membership in the just community, and affirms our ongoing concern for the living. The procedure is trusted and tried and has moved out of the realm of experimental medicine. Maintaining the brain dead briefly in order to harvest organs critically needed for the living is clearly justified.

Maintaining the brain dead for prolonged periods as incubators for potential others is not without inherent problems. The other we seek to enhance is a potential being; the procedure and its results have little precedent to guide us and, furthermore, are enormously time and resource consuming. While, symbolically, the potential being is the "flesh of the flesh" and the "life of the life" of the person that just recently ceased to be, things here stand differently than when the newly dead are briefly maintained to salvage organs.

Moral differences between early and late-stage pregnancies

Our attitude toward the question of moral differences between early and late-stage fetuses directly involves our attitudes toward abortion. However, unlike the abortion argument, an agreement can hope to be achieved. In the abortion argument, the attitude basing its viewpoint on immediate ensoulment or personhood of the newly conceived stands in opposition to the attitude that holds that the interests of the developing fetus (at least prior to central nervous system function) have less force than those of the mother. Here is not the time or place to engage in this interminable and probably fruitless discussion. In general, however, both sides would agree that the embryo, far from being without interests and thus being purely an object to be acted on, has inherent interests. The two sides disagree since one holds the interests of the fetus to be equivalent to those of the mother, whereas the other holds them to have considerably lesser force.

In the case under discussion, the primary interests of the mother, by definition, cannot be invoked; they either do not exist or, at the very most, stand permanently moot. Her value and interests are now secondary (as she is of value to others) and symbolic. This value and these interests stand balanced with those of the developing fetus whose interests, at the very least, are potential and growing.

There is one critical difference, whether one does or does not hold all human life to have "absolute" rights. The current state of the art makes it possible, even if

very difficult, for us to achieve success in producing a viable infant from a brain-dead mother at 24 weeks and remotely possible but excruciatingly difficult to achieve the same results at 22 weeks; the possibilities are entirely unknown earlier than that. Further, while it is quite possible to have not only a viable but also a "normal" fetus at 24 weeks, experience earlier than that is either sparse or entirely absent. That changes the dimensions of the problem.

Resolution

The situation that confronts physicians and institutions today has changed markedly from what it was a few years ago. So has the moral climate. On the one hand, technical advances have enabled physicians to seek their patients' good in much more forceful ways; on the other, that "good" is no longer properly decided by physicians or institutions but is the product of informed deliberation in which all those directly concerned partake. Choices are no longer the sole responsibility of a physician working in a particular institution under its autonomously determined rules. Medicine, as well as the institutions in which medicine operates, has properly become a communal venture, forced to a large extent by economic conditions.

The current problem is not one open to easy, facile, or permanent solutions. Attitudes will and must depend on the feasibility, probability, and, yes, ease of the solutions sought. Stopping support of the brain-dead mother, thereby inevitably causing the death of the fetus, is analogous to abortion only insofar as the fetus is either viable at that time or close enough to viability so that its survival can be reasonably assumed; the farther away from that time it is, the more it is unlike abortion.

Resolution in concrete situations will, to a large extent, depend on the available technology. Feasibility depends entirely on this and, unless a procedure is at least feasible, its moral standing is moot. The probability of success is another variable that strongly affects our approach. An action whose likelihood of success is great (for example, delivering a baby by cesarean section at term from a mother whose heart has just stopped) is an entirely different matter than one unlikely to succeed or one whose outcome is virtually unknown. Further, success here begs definition: Do we mean the delivery of a normal, viable infant or are we willing to settle for a severely damaged one? When an intervention has not been routinely applied, when the venture is fraught with unknown problems ahead, and when risk/benefit ratios remain undefined, the procedure is generally held to be experimental. Experimental procedures require the most careful informed consent. Such consent is "a statement of fidelity between the man who performs medical procedures and the man on whom they are performed."[14] When that one is unable to speak for himself/herself, the next of kin as surrogate traditionally has spoken. The very definition of "informed" is difficult since, by the nature of the undertaking, the person informing often has only little information to give. Outcomes, risks, and benefits are nebulous. Often previous trials, animal experiments, or analogous precedent may tentatively help but "hard" information, by the very definition of the undertaking, which is a venture into the unknown, is unavailable. At times, the best physicians can do is to share their honest ignorance, their concerns, and their doubts. The current problem has little concrete precedent and, at best, there are only a few anecdotal examples to light the way. These are examples of success; failures, by their very nature, usually remain unpublished.

Unless one feels comfortable with late third-trimester abortion, there is little doubt that when the pregnancy is well advanced, for instance, beyond 28 weeks, immediate delivery by cesarean section is indicated. If one finds no moral problems in the abortion of viable infants, that statement may seem less obvious. For the sake of this article, I will maintain that our current communal ethos is sufficiently opposed to the destruction of fully viable infants (except, perhaps, under the most severe conditions of threat to the life of the mother) as to make such a solution not tenable. It follows that when viability is reasonably assured and when delivery does not threaten the life of the mother, delivery should be a matter of routine medical treatment. At 28 weeks and beyond, there is a fair chance for success and a reasonable hope that the infant will be normal. Treatment then is no longer experimental and, barring unusual circumstances, must proceed.

When gestation is shorter, the attempt to maintain brain-dead mothers as incubators for their developing offspring would need to be carefully discussed with the next of kin and would require informed consent. As the length of gestation diminishes, so does our ability to predict outcomes. Procedures more and more approach the innovatively experimental and therefore more and more require consent. That consent, in the nature of things, will be less and less able to be "informed" and more and more speculative. No moral mandate can enjoin one to subject one's patient to experimental therapy; no moral mandate can force patients to accept such treatment. Not maintaining a brain-dead mother as an incubator for her infant in early pregnancy, for instance, before the twentieth week, is no more like abortion than not giving experimental chemotherapy to a dying cancer patient is murder. Maintaining the brain dead as incubators (or giving experimental chemotherapy) is morally permissible when proper consent (consent that makes clear the experimental nature of the undertaking) is obtained, but it certainly is not a moral imperative.

In individual cases, judgments will depend greatly

on the circumstances. When a patient is in her first trimester, when the fetus is, in some probability, assumed to have been severely damaged by the mother's abnormal condition (sustained hypoglycemia may well be such a case), or when other formidable technical problems are present, an attempt to maintain the brain-dead mother as incubator seems, in the current state of medicine, ill advised. As 20 weeks is approached, the possibility of success is ever better even if its likelihood continues to be rather remote. At this point, earnest conversation with the next of kin (rather than a "sportive" attempt to prove a point and to add another case of the "Annals of Unlikely Outcomes") is in order. Here the factors of cost, material and emotional, must have a place. An about to be bereaved father of three children may be in no position to underwrite or undertake an experimental venture that then leaves him with one more child to raise alone. The community has no right to try to persuade the next of kin to participate unless perhaps the community is, at the very least, ready to assume the financial burden of the venture as well as of its consequences. Even then, it is still the next-of-kin's choice and not ours to judge.

At about 24 weeks and with at least some precedent of success, there is still a need for informed consent. Here, where information is at least more available, suggesting to the relatives that an attempt may be worthwhile seems reasonable. Nevertheless, the community and the institutions that represent it should, in decency, stand ready to assume at least the financial burden. Communal duties to assist those struck by disaster or tragedy are, however, another matter and legitimately a separate topic.

Resolution of these tragic cases cannot be made without attention to the situation, its context, and the moral actors involved. Strict criteria denoting moral certitude here, as in most of life, of which medical ethics is but a part, cannot be applied and ambiguity inevitably persists.[15] Denying this reduces ethics to yet another strictly technical exercise that seeks to "apply" ready-made answers to preconceived problems without filtering them through our moral sensibilities. Decisions in particular cases must rely on an interplay among moral actors and their peculiar contexts. In the final analysis our decisions reveal more about us and about the other moral actors involved than they do about the problems themselves. Discretion is the essence of decision making and discretion proceeds in a context and within a historical time frame. Not ultimately and forever right or wrong, decisions represent our groping for truth rather than truth itself. Their moral virtue resides in the agony of decision making rather than in the particular decision itself.

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Unintended laparotomy associated with laparoscopic tubal sterilization

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Although the complication rate for laparoscopic tubal sterilization is generally low, laparotomy is sometimes necessary to complete the sterilization or manage complications. To better characterize factors that predispose women to unintended laparotomy, we analyzed data from the Collaborative Review of Sterilization. Of the 5027 women undergoing laparoscopic tubal sterilization, 12 had unintended laparotomies to manage complications, whereas 39 women had unintended laparotomies because of technical inability to complete the laparoscopic procedure. Women with prior abdominal or pelvic surgery had an increased risk of unintended laparotomy (relative risk = 10.2, 95% confidence interval = 5.3 to 19.7). Women with a history of intrauterine device use or pelvic inflammatory disease had elevated risks that were not statistically significant (relative risk = 2.2 and 1.5, respectively). Comparative studies of alternative surgical approaches for tubal sterilization are needed to formulate recommendations for women who may be at increased risk of unintended laparotomy associated with the laparoscopic approach. (AM J OBSTET GYNECOL 1987;157:1102-5.)

Key words: Laparoscopic tubal sterilization, laparoscopy complications, tubal sterilization methods

Since 1970 sterilization via laparoscopy has increased dramatically in popularity and now accounts for at least one half of all nonpregnancy-associated tubal sterilization procedures in the United States.[1] The laparoscopic approach has proved safe and effective overall and involves shorter hospital stays than traditional laparotomy.[2] Under some circumstances, however, the sterilization procedure cannot be completed laparoscopically because of technical difficulties or arising complications, which necessitate a laparotomy. Such unintended laparotomies expose women to the surgical risks of both procedures, prolonged anesthesia, and in some cases a second anesthetic and hospitalization.

Several studies have implicated previous abdominal or pelvic surgery, a history of intrauterine device (IUD) use, or pelvic inflammatory disease as possible risk factors for intraoperative difficulties or unintended laparotomy.[3-6] A preliminary analysis of laparoscopic sterilization complications that used the first 3500 women enrolled in the Collaborative Review of Sterilization has been previously published.[7] Results suggested that previous surgery and pelvic inflammatory disease modestly increased the risk of complications, the most common of which was unintended laparotomy.

None of the previous studies have assessed all three of these potential risk factors simultaneously. To better characterize unintended laparotomy and assess these three risk factors, we used data from more than 5000 women enrolled in the Collaborative Review of Sterilization, a multicenter prospective study of the safety and efficacy of female sterilization operations.

Table I. Reasons for unintended laparotomy associated with interval laparoscopic tubal sterilization, CREST 1982

	No.
Technical problems	
Adhesions/anatomic distortion	32
Difficulty with trocar, insufflation	7
Total	39
Complications	
Perforated organs	4
Hemorrhage	4
Other*	4
Total	12
Total unintended laparotomies	51

CREST = Collaborative Review of Sterilization.
*Includes one case each of peritonitis, pyosalpinx, umbilical hernia, and abdominal pain.

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Table II. Relative risk of unintended laparotomy by preoperatively known risk factors, women undergoing laparoscopic tubal sterilization, Collaborative Review of Sterilization 1982

Preoperative risk factors	*No. of UL*	*No. of women*	*Rate (%)*	*RR*	*95% CI*
None	8	2377	0.3	1.0	(Referent)
Previous surgery only	21	610	3.4	10.2	(5.3-19.7)
PID only	1	196	0.5	1.5	(0.2-11.9)
IUD only	9	1192	0.8	2.2	(0.9-5.7)
>1	12	652	1.8	5.5	(2.5-12.1)

UL = Unintended laparotomy; RR = rate ratio, or relative risk, comparing each risk group with group without risk factors; CI = confidence interval; PID = pelvic inflammatory disease.

Material and methods

From 1978 to 1982, 5342 women scheduled for laparoscopic tubal sterilization were enrolled from 11 institutions in five cities. All women were 15 to 44 years old and were not pregnant on admission to the medical center. Before the operation, each woman was interviewed with a standard questionnaire. The interview included questions regarding medical history and previous contraception. In our analysis previous surgery was defined as a patient report of any prior surgery involving the abdomen or pelvis. Previous pelvic inflammatory disease was defined as a patient report of having been diagnosed with pelvic inflammatory disease by a physician. Previous IUD use was defined as a patient report of having used an IUD in the past.

Information regarding surgical details was recorded intraoperatively.

Postoperative information was obtained from the hospital record, the first follow-up interview approximately 4 weeks later, and the second follow-up interview at 1 year. Unintended laparotomy was defined as laparotomy within 6 weeks of the laparoscopic procedure, which was performed to manage complications from the laparoscopy or to complete the sterilization.

This definition excluded laparotomies performed for the treatment of pathologic conditions observed at laparoscopy (n = 5), for unrelated disorders (n = 3), and because of equipment malfunction (n = 1). Also excluded were 265 women who underwent concurrent operative procedures (such as ovarian biopsies or drainage of ovarian cysts) that could have led to complications. Dilatation and curettage, routine IUD removal, or lysis of adhesions were not considered concurrent procedures for the purpose of exclusion. Also excluded were 41 women whose histories were incomplete regarding previous surgery, IUD use, or pelvic inflammatory disease. After exclusions, the cohort included 5027 women.

Crude relative risks and test-based confidence intervals were calculated by means of the method of Miettinen.[8] Where the confidence interval does not include 1.0, the results are statistically significant at the $p < 0.05$ level. Logistic regression methods[9] were used to assess potentially confounding effects of age, race, intended method of tubal occlusion, type of anesthesia (local versus general), and obesity.

Results

Fifty-one women from this cohort underwent an unintended laparotomy, which resulted in an overall rate of 1.0%. Thirty-nine laparotomies were performed because of technical problems with the attempted laparoscopic procedure, whereas 12 were performed to manage complications.

Of the 39 laparotomies performed because of technical problems (Table I), 32 resulted from encountering adhesions, hydrosalpinx, or other anatomic distortions, whereas seven resulted from difficulty entering the peritoneal cavity. Among these 39 women, 35 had the laparotomies under the original general anesthesia. Compared with women who did not have a laparotomy, anesthesia time was prolonged (mean 83 versus 26 minutes), and the rate of postoperative complications (fever, transfusion, rehospitalization, and life-threatening event) was increased (11.4% versus 0.6%). The remaining four women underwent laparotomy during a subsequent hospitalization under a second anesthetic (details unavailable).

Among the 12 women with laparoscopic complications (Table I), two required total abdominal hysterectomies and three required bowel resections. A second hospitalization and second anesthetic was necessary in the treatment of six of these 12 women.

Overall, 21% of the women in the entire cohort had a history of prior abdominal or pelvic surgery whereas 35% had a history of IUD use and 10% had a history of pelvic inflammatory disease. These are not exclusive categories. Forty-seven percent of women had none of these potential risk factors.

For both complications and technical problems, the unintended laparotomy rate was higher for women with any of the risk factors than for women without these risk factors. Overall unintended laparotomy rates for each exclusive risk group (Table II) were as follows:

3.4% for previous surgery, 0.8% for IUD use, 0.5% for pelvic inflammatory disease, and 0.3% for women with none of these risk factors. Women with more than one of these risk factors had an unintended laparotomy rate of 1.8%.

The relative risk, or rate ratio, of unintended laparotomy for women with a history of prior abdominal or pelvic surgery relative to women with no risk factors was 10.2 (95% confidence interval = 5.3 to 19.7). For women with a history of IUD use or pelvic inflammatory disease, the increased relative risks of 2.2 and 1.5, respectively, were not statistically significant.

Multivariate analysis adjusting for age, race, intended method of tubal occlusion, anesthesia, and obesity yielded no appreciable alteration in relative risks associated with the three risk factors under investigation.

When a history of prior cesarean section (32% of women with previous surgery as their only risk factor) was evaluated separately from other abdominal or pelvic surgery, the rates of unintended laparotomy were 5.9% for cesarean section only and 2.4% for other surgery only. Compared with women with no risk factors, the risk ratios were 17.5 (95% confidence interval = 8.8 to 34.8) and 7.1 (3.2 to 15.8), respectively. Information was unavailable regarding types of abdominal or uterine incisions for these cesarean sections.

Comment

We addressed the hypothesis that conditions that may predict anatomic abnormality, such as adhesions or adnexal disease, increase the risk of unintended laparotomy as an outcome of laparoscopic tubal sterilization. Our finding of a 10-fold increase in risk for women with previous abdominal or pelvic surgery is consistent with previous studies and helps quantitate long-held clinical suspicion. This elevated risk pertains to women with previous cesarean sections as well as other operations of the abdomen or pelvis.

IUD use was also associated with an increased risk. Although this increase was not statistically significant, our findings are consistent with biologically plausible effects. Among the 1192 women who had only a history of IUD use but did not have pelvic inflammatory disease or prior surgery, nine had unintended laparotomies. Five of these were necessary because of abnormalities consistent with subclinical pelvic inflammatory disease, such as hydrosalpinx.

We believe that the risk associated with pelvic inflammatory disease may have been underestimated because patient histories may not be reliable indicators of previous pelvic inflammatory disease.[10] Furthermore, women who recalled the clinical diagnosis of PID may have been more likely to have received treatment than women who did not. If treatment diminishes pelvic sequelae, the women classified as having pelvic inflammatory disease in our cohort may have been less at risk for unintended laparotomy than women who actually had pelvic inflammatory disease but did not recall it.

In summary, data consistently show that some women are predictably at higher risk of technical problems or complications during laparoscopic tubal sterilization. Whether any alternative surgical approach can be recommended for them remains unresolved. There is currently some enthusiasm for two less commonly used surgical approaches: open laparoscopy,[11] which is done entirely under direct vision, and minilaparotomy,[12-14] which involves a small (2 to 5 cm) laparotomy incision. Although both of these procedures may seem promising to some, insufficient epidemiologic data exist to compare them with traditional laparoscopy or laparotomy.

Therefore the important question of whether these procedures offer any advantage to women with abdominal or pelvic abnormalities cannot presently be answered. To address this question will require study of a large number of tubal sterilizations performed by these alternative techniques. In the absence of comparative studies, it is not possible from the epidemiologic data to issue recommendations for women at higher risk of unintended laparotomy. Our data provide an estimate of the magnitude of the increased risk, and leave the choice of surgical approach a matter for the individual clinician and patient to consider.

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Decreasing incidence of invasive cervical cancer in young women

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Although the overall incidence of invasive cervical cancer is decreasing, there is concern that the disease might be increasing in younger women. Analysis of data from the Cancer Surveillance System, a population-based cancer registry in western Washington, shows a decreasing incidence of cervical cancer in women of all ages. (AM J OBSTET GYNECOL 1987;157:1105-7.)

Key words: Cervical cancer, incidence in young women

Although the incidence of invasive cervical cancer has significantly decreased over the past decade in most Western countries, there is concern over the possible increase in occurrence and mortality in young women.[1-7] Possible reasons for this increased incidence and mortality in young women include increased sexual activity and infections, such as papillomavirus.

In 1978 Yule[1] noted that the number of deaths from cervical cancer in women under 35 years of age living in England and Wales had doubled between 1970 and 1976. This increase over time is quite different from the decrease seen in older women with cervical cancer. Similarly, there was an increase in the incidence of invasive cervical cancer among women aged 20 to 44 years between 1974 and 1980 in Queensland, Australia.[2]

Because of these observations in other geographic areas and the possible implications for cancer control, we examined the data from the Cancer Surveillance System of western Washington during two recent periods.

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Methods

Since 1974 the Cancer Surveillance System has been part of the National Cancer Institute's population-based registry program (SEER). The Cancer Surveillance System collects incidence data on all newly diagnosed malignancies in the Puget Sound area from all hospitals, radiation therapist offices, and pathology laboratories. For our study, information for all cases of newly diagnosed invasive cervical cancer from 1974 to 1985 was abstracted from the registry. Annualized age-specific incidence rates were calculated for two 6-year periods, 1974 to 1979 and 1980 to 1985, on the basis of denominator estimates of the 1970 and 1980 census, and from population projections made by the U.S. Bureau of Census and the Washington State Office of Financial Management.

Results

Similar to other geographic areas, there was an overall decrease in the incidence of invasive cervical cancer, from 10.8/100,000 in 1974 to 1979 to 7.9/100,000 in 1980 to 1985 in western Washington (Table I). In ad-

Table I. Annualized age-specific incidence rates (per 100,000 women) of invasive and in situ cervical cancer in western Washington

Age at diagnosis (yr)	*Invasive (year of diagnosis)*					*In situ (year of diagnosis)*				
	1974-1979		*1980-1985*		*Percent of decrease (increase)*	*1974-1979*		*1980-1985*		*Percent of decrease (increase)*
	Rate	*n*	*Rate*	*n*		*Rate*	*n*	*Rate*	*n*	
All women	10.7	865	7.9	779	26	56.2	4944	50.5	5493	10
20-24	2.2	16	2.1	16	5	93.5	634	114.5	887	(22)
25-29	10.0	67	8.6	70	14	207.9	1405	181.5	1479	13
30-34	16.1	91	11.4	88	29	197.7	1143	165.1	1283	16
35-39	19.8	89	14.1	93	29	144.7	646	118.8	772	18
40-44	21.2	81	17.2	85	19	99.7	384	78.5	385	21
45-49	22.0	85	12.8	52	42	58.7	226	52.8	215	10
50-54	20.2	82	14.8	59	27	33.2	135	27.3	98	18
55-59	16.9	63	16.2	68	4	24.5	93	18.9	79	23
60-64	23.8	77	14.0	56	41	18.8	61	18.7	75	1
65-69	26.6	75	18.5	62	30	23.0	64	17.7	50	23
70-74	24.6	54	15.2	41	38	18.1	39	7.4	20	59
75-79	16.3	27	19.3	39	(18)	13.8	23	7.9	16	43
80-84	26.6	32	18.6	26	30	7.6	13	11.5	16	(51)
≥85	26.5	25	16.4	21	38	6.5	6	4.7	6	28

dition to the decreasing incidence in the older age group, however, we also see a decreasing incidence in all 5-year age groups of younger women (those 39 years old or younger) in this area. It should be noted, however, that the percent decrease is less in women under 30 years of age.

Additional analyses were performed to investigate any possible changes in mortality in younger women. The stage at diagnosis and the survival of women with cervical cancer did not show any indication of increasing mortality in younger women.

Comment

Our finding of a decreasing incidence of invasive cervical cancer is contrary to "clinical impression." We do not believe our results or our geographic area are atypical, because our findings are very similar to those from other areas of the United States. Chow et al.[8] found a decreasing incidence of invasive cervical cancer in white women in Atlanta between 1975 and 1983 in women aged 25 to 29 and 30 to 34 years but not in women 15 to 24 years old. Peters et al.[9] also noted an annual decrease of 2.5% in invasive squamous cell carcinoma of the cervix in women <35 years old between 1972 and 1982 in Los Angeles. Analysis of data from the National Cancer Institute's 10 population-based SEER registries (approximately 10% of the U.S. population) shows a 16% decrease (from 5.8 to 4.9/100,000) in invasive squamous cancer of the cervix in women ages 15 to 39 years between 1974 to 1977 and 1982 to 1984.

Although the number of women diagnosed with invasive adenocarcinoma of the cervix is relatively small, the incidence of this disease in women <35 years old has increased.[9, 10] Schwartz and Weiss[10] noted over a twofold increase in women <35 years old between 1973 to 1975 and 1979 to 1982 (0.17 to 0.38/100,000) in the SEER program.

There are several explanations why our findings might differ from those of other studies.[1-6] All of the studies except one[4] that showed an increase in number of cases, incidence, or mortality among young women with cervical cancer are from outside the United States. The frequency of cytologic screening is quite different, and prevalence rates of the various risk factors might be different in these countries than the United States. Second, most of the reported increases in deaths and mortality rates in younger women with cervical cancer occurred in the 1960s and 1970s. Our registry began in 1974; thus it is possible that any increase before the beginning of our registry was missed.

Other factors might impair our ability to determine the incidence of invasive cervical cancer. First, the possibility that cases of invasive cervical cancer are not being ascertained is small. Case ascertainment is conducted through active search in multiple souces of data. The Cancer Surveillance System sends trained abstractors to all hospitals and radiation facilities to review hospital disease and operation indices and pathology reports. In addition, death certificates are checked. Second, if the pathologic criteria for diagnosing invasive cervical cancer had changed during the study period, it would be possible to find an artificial decrease. However, there has not been any change in the pathologic criteria for invasive cervical cancer during this time period. Third, if there were intensive screening of

young women, perhaps fewer women ever reach invasive cancer but instead are diagnosed with preinvasive stages such as dysplasia or carcinoma in situ. Consistent with this is the high rate of carcinoma in situ in our area. However, time trends of carcinoma in situ in most 5-year age groups in our area show decreases similar to those of invasive cancer (Table I). This decrease needs to be viewed with some caution, because it is likely that many cases recently diagnosed and treated have had all their care as outpatients and therefore may have not been ascertained. The findings of declining rates of carcinoma in situ have also been observed in Atlanta[8] and in Los Angeles.[9] Unfortunately, we do not know whether the incidence of less severe dysplasias (cervical intraepithelial neoplasia I and II) is increasing or decreasing, because the Cancer Surveillance System does not collect this information.

Our results, as well as those from other area-wide registries in the United States, do not support the clinical impression or previously published studies from outside the United States that there is an increase in the overall incidence of invasive cervical cancer in young women. This does not mean that the actual number of cases of invasive cervical cancer in young women is decreasing, because there are now more young women in the population. Whether the incidence of invasive cervical cancer will increase with the observed increases in papillomavirus infections of the cervix needs to be closely followed.

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Menstrual and reproductive factors and the risk of myocardial infarction in women under fifty-five years of age

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The relationship between menstrual and reproductive factors and subsequent risk of coronary heart disease was investigated in a hospital-based case-control study of 202 women with acute myocardial infarction and 374 control subjects admitted for a wide spectrum of acute conditions unrelated to any of the established risk factors for ischemic heart disease. No consistent association was observed with age at menarche or menopausal status, but women with a lifelong irregular menstrual cycle pattern were at significantly elevated risk of myocardial infarction (relative risk = 1.8, 95% confidence interval = 1.1 to 2.9). No clear trend in risk was evident with the number of livebirths, miscarriages, or induced abortions. However, women whose first pregnancy or livebirth occurred before age 20 years showed elevated risks of subsequent myocardial infarction compared with nulliparous ones (relative risks = 2.3; 95% confidence interval = 1.1 to 4.9), and there was a significant trend of increasing risk with earlier first birth. These associations were evident in both younger and middle-age women and were not explained by allowance for several identified potential confounding factors. (Am J Obstet Gynecol 1987;157:1108-12.)

Key words: Myocardial infarction, menstrual and reproductive factors, pregnancy

Evidence indicates that female hormones influence the risk of ischemic heart disease. The use of oral contraceptives increases the risk of myocardial infarction,[1] whereas it is still debated whether menopausal estrogen replacement treatment decreases the risk of cardiovascular disease.[2-4]

Fewer data are available on the potential role of endogenous hormones and their correlates, that is, menstrual and reproductive factors. Although it is known that the advantage that women have over men in the incidence of coronary heart disease and death is confined to the premenopausal years (postmenopausal female rates rise to become comparable with those for elderly men[5]), it is still uncertain whether early menopause increases the risk of myocardial infarction relative to premenopausal women of comparable age.[6-8] With regard to reproductive history, a study based on the Mayo Clinic record linkage system showed elevated risks in women whose first pregnancy was below age 25 years,[9] but the evidence was controversial in a large case-control study conducted in three geographic regions of North America.[8] In the present study, we have further considered the role of menstrual and reproductive factors in the risk of myocardial infarction with data from a case-control study conducted in Northern Italy where mortality rates from ischemic heart diseases are considerably lower than those in Northern European or American populations.[10]

Subjects and methods

Since January 1983, trained interviewers identified and questioned women below age 55 years admitted for acute myocardial infarction to the coronary care units of 30 hospitals in Lombardy and a few other areas in northern Italy (Turin, Genova, Verona, and Pisa). The general design of this investigation has already been described.[11] Briefly, a regular liaison with cooperating hospitals was maintained at approximately fortnightly intervals. Less than 2% of the case or control subjects approached for interview refused to participate.

A standard questionnaire was used to obtain information on sociodemographic factors, personal characteristics and habits (i.e., cigarette smoking, consumption of alcohol, coffee, and other methylxanthine-containing beverages), a few selected dietary items, menstrual and reproductive factors (i.e., age at menarche, menopausal status, lifelong menstrual pattern, number of livebirths and abortions, and age at first pregnancy and at first and last birth), and history of

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Table I. Distribution of 202 cases of acute myocardial infarction and 374 control subjects according to selected risk factors, Northern Italy, 1983 to 1986

Risk factor	*Myocardial infarction*		*Controls*	
	No.	*(%)*	*No.*	*(%)*
Age (yr)				
<40	35	(17.3)	133	(35.6)
40-49	82	(40.6)	134	(35.8)
50-54	85	(42.1)	107	(28.6)
Cigarette smoking				
Never	63	(31.2)	245	(65.5)
Exsmoker	6	(3.0)	17	(4.5)
Current	133	(65.8)	112	(29.9)
Diabetes				
No	179	(88.6)	358	(95.7)
Yes	23	(11.4)	16	(4.3)
Hypertension				
No	120	(59.4)	339	(90.6)
Yes	82	(40.6)	35	(9.4)
Hyperlipidemia				
No	162	(80.2)	348	(93.0)
Yes	40	(19.8)	26	(7.0)
History of coronary heart disease in first degree relatives				
No	76	(37.6)	208	(55.6)
Yes	126	(62.4)	166	(44.4)

lifetime use of oral contraceptives and female hormones for other indications.

The cases studied included 202 women under 55 years of age with a diagnosis of acute myocardial infarction according to the standard World Health Organization criteria. An additional 18 patients were considered unsuitable after review of the clinical records, chiefly because of recurrent infarction (n = 10) or uncertain diagnostic criteria (n = 8). The median age was 47 years, and 117 (58%) women were below age 50 years.

The control subjects were patients under age 55 years admitted to the same hospitals where cases had been identified for acute diseases other than cardiovascular, malignant, metabolic, hormonal, or gynecologic, which were diagnosed within the year before interview. Further exclusion was made of all primary diagnoses potentially related to cigarette smoking (e.g., peptic ulcer or abdominal hernia) or alcohol consumption (e.g., liver diseases). A total of 374 women 24 to 54 years old were interviewed; the median age was 45 years, and 267 (71%) were below age 50 years. Among them, 22% were admitted for traumatic conditions (mostly fractures or sprains), 22% had nontraumatic orthopedic disorders (mostly lower back pain and disc disorders), 25% were admitted for surgical conditions (including plastic surgery), and 31% had other illnesses, such as acute infections, skin, ear, nose and throat, or teeth disorders. The present analysis is based on data collected before February 1986.

Data analysis. Since cases were older than the comparison group, relative risk estimates (together with their 95% approximate confidence intervals)[12] were derived from data stratified for quinquennia of age by means of the usual Mantel-Haenszel procedure.[13] The significance of the linear trends in risk, where appropriate, was assessed with the test described by Mantel.[14] The potential confounding effect of several factors (geographic area, marital status, education, social class, cigarette smoking, alcohol and coffee consumption, diabetes, hypertension, obesity, hyperlipidemia, family history of ischemic heart disease, oral contraceptive and other female hormone use) was controlled for by means of stratification and the Mantel-Haenszel procedure.[13] Second, identified potential distorting factors were simultaneously controlled for by multiple logistic regression, fitted by the method of maximum likelihood.[12] Included in the regression equations, in addition to the menstrual and reproductive factors considered in this article, were terms for age, geographic area, marital status, education, social class, cigarette smoking, alcohol consumption, diabetes, hypertension, obesity, hyperlipidemia, family history of ischemic heart disease, and oral contraceptive and other female hormone use. Relative risk estimates from multiple logistic models were not materially different from the age-adjusted Mantel-Haenszel estimates, and only the latter are presented in the tables.

Results

The distribution of cases and control subjects according to age and risk factors showing significant as-

Table II. Distribution of 202 women with acute myocardial infarction and 374 control subjects according to selected menstrual characteristics

	Myocardial infarction	*Controls*	*Relative risk estimates (95% CI)**
Age at menarche (yr)			
<12	55	62	1†
12-14	110	258	0.49 (0.31-0.75)
≥15	35	54	0.61 (0.33-1.12)
Unknown	2	—	
Menstrual cycle pattern			
Regular	161	320	1†
Irregular‡	40	52	1.79 (1.13-2.86)
Unknown	1	2	—
Menopausal status			
Before or during	117	252	1†
After natural	63	79	0.77 (0.44-1.35)
After surgical	22	43	0.69 (0.39-1.23)

CI = Confidence interval.

*Mantel-Haenszel estimates adjusted for age in quinquennia.

†Reference category.

‡Defined as frequent occurrence of menstrual-like episodes less than 21 or more than 35 days apart.

Table III. Distribution of 202 women with acute myocardial infarction and 374 control subjects according to number of livebirths and abortions

	Myocardial infarction	*Controls*	*Relative risks estimates (95% CI)**
No. of livebirths			
0	35	123	1†
1-2	129	156	1.85 (1.15-2.96)
≥3	38	95	0.75 (0.40-1.39)
No. of miscarriages			
0	147	293	1†
1	37	54	1.03 (0.69-1.74)
≥2	18	27	1.08 (0.56-2.06)
No. of induced abortions			
0	181	331	1†
≥1	21	43	0.96 (0.54-1.70)

CI = Confidence interval.

*Mantel-Haenszel estimates adjusted for age in quinquennia.

†Reference category.

sociation with the risk of myocardial infarction is shown in Table I. Women with myocardial infarction more frequently were smokers and reported a history of diabetes, hypertension, hyperlipidemia, and a family history of coronary heart disease.

In Table II the relationship between selected menstrual characteristics and the risk of myocardial infarction is considered. Compared with women whose menarche occurred younger than 12 years, those with later menarche showed a reduced risk of infarction. However, there was no tendency for the risk to decrease with increasing age at menarche. Women with lifelong menstrual irregularities (simply defined as frequent occurrence of menstrual-like episodes less than 21 or more than 35 days apart) were at significantly elevated risk (relative risk = 1.8, 95% confidence interval = 1.1 to 2.9). In contrast, when age was allowed for, no significant relationship was evident between menopausal status and myocardial infarction risk. Allowance for major identified potential distorting factors by means of multiple logistic regression did not materially modify any of these estimates (i.e., multivariate relative risk for irregular menstrual cycle pattern = 2.1, 95% confidence interval = 1.2 to 3.3).

There was no consistent pattern of risk according to the number of livebirths, since the point estimate was above unity for women with one or two children and below unity for those with three or more births compared with nulliparas (Table III). Likewise, there was no relationship between infarction risk and the number of miscarriages or induced abortions (Table III).

Relative to women who had never been pregnant, the risk was significantly elevated (relative risk = 2.3, 95% confidence interval = 1.1 to 4.9) for those whose first pregnancy was below age 20 years, and the point estimate was above unity, although not significantly (relative risk = 1.5), for those women whose first pregnancy occurred above age 20 years (Table IV). The pattern of risk was similar with regard to age at first livebirth, and a significant trend of increasing risk with younger age at first livebirth was evident (Table IV). The relationship between early first birth was independent from the major identified potential distorting factors, since the multivariate relative risks were not materially different from the age-adjusted ones (relative risk for women before 20 years old at the first birth = 2.4 and 1.4 for those aged 20 to 24 years).

Likewise, the risk of myocardial infarction was significantly elevated (relative risk = 2.1) in women whose last birth occurred below age 25 years. However, the data are insufficient to allow separate analysis of the effects (and the interactions) of parity and age at first and last birth, which are, of course, strongly correlated.

The influence of early births on subsequent myocar-

dial infarction risk was long lasting, since the risk estimates were above unity for middle-aged (45 to 54 year) women, as well as for younger ones.

Comment

The findings of the present study indicate that several aspects of menstrual and reproductive history are related to the subsequent risk of acute myocardial infarction in younger and middle-aged women. The relative risk was significantly elevated for women with frequent irregularities in menstrual cycle and those with earliers pregnancies or births. No consistent association was evident for age at menarche, menopausal status, parity, and number of abortions.

It is unlikely that the present findings are largely influenced by obvious bias. The control subjects were admitted for a wide spectrum of acute conditions requiring hospital treatment apparently unrelated to menstrual or reproductive events, and the participation rate was almost complete. Several variables considered (parity, age at first birth, or menopausal status) should not be influenced by recall or information bias, and also for variables whose recall may not be totally reliable (i.e., age at menarche or menstrual cycle pattern), there is little support for different recall by cases and control subjects having any considerable impact on the risk estimates. The potential influence of menstrual and reproductive factors on myocardial infarction risk, in fact, was not known to the interviewers and probably not to most patients either, and the major attention in this study was on the effects of other risk factors, such as exogenous female hormones or smoking.[11]

With regard to confounding, the main findings of this study were not materially influenced by allowance for several identified potential distorting factors, including sociodemographic indicators and the major risk factors for myocardial infarction.

Thus this study confirms the indication of a case-control study based on 169 incidences of coronary heart disease (angina, myocardial infarction, and sudden unexpected death) in women under age 60 years from Rochester, Minnesota, during 1960 to 1979, which showed a relative risk of 1.9 for women whose age at first pregnancy was under 20 years and 1.8 for those 20 to 24 years compared with never pregnant women and those with later first pregnancy.[9] The results of another large North American case-control study were more controversial and not statistically significant, although the point estimates for women whose first pregnancy was below age 25 years were also above unity in that study.[8]

A second interesting finding of this study was the elevated risk among women with lifelong irregular menstrual cycle pattern. Although the rather crude definition used for "menstrual irregularity" does not allow precise definition of the biologic correlates of this finding, it is likely that women with menstrual irregularities have more frequent anovular cycles and several steroid hormone imbalances (i.e., hyperandrogenemia). It is, however, still difficult to reconcile in unifying and widely accepted hormonal correlates the various findings of the present study, including the absence of association with menopausal status (which is in agreement with some but not all previous studies).[6-8] Likewise, other pathogenetic hypotheses put forward to explain the lower cardiovascular mortality rates in women based, for instance, on the suggestion that menses may provide a mechanism for the elimination of blood-borne factors that participate in atherogenesis[15] are partly (i.e., positive association with irregular menses) but not totally (i.e., absence of association with age at menopause) consistent with the findings of the present study.

The influence of the major risk factors in the present

Table IV. Distribution of 202 women with acute myocardial infarction and 379 control subjects according to age at first pregnancy and at first and last livebirth

	Myocardial infarction	*Controls*	*Relative risk estimates (95% CI)**
Age at first pregnancy (yr)			
<20	24	28	2.31 (1.10-4.86)
20-24	76	132	1.52 (0.89-2.59)
≥25	73	106	1.55 (0.86-2.82)
Nulligravidas	29	108	1†
χ_1^2 (trend)			3.30 (p = 0.07)
Age at first livebirth (yr)			
<20	21	23	2.31 (1.10-4.87)
20-24	71	117	1.47 (0.89-2.44)
≥25	75	111	1.39 (0.80-2.41)
Nulliparas	35	123	1†
χ_1^2 (trend)			3.99 (p = 0.05)
Age at last livebirth (yr)			
<25	39	43	2.14 (1.15-3.97)
25-29	47	87	1.09 (0.60-1.98)
≥30	81	121	1.30 (0.75-2.24)
Nulliparas	35	123	1†
χ_1^2 (trend)			4.15 (p = 0.04)

CI = Confidence interval.

*Mantel-Haenszel estimates adjusted for age in quinquennia.

†Reference category.

study (irregular menstrual pattern and age at first pregnancy or birth) was still evident in middle-aged women, that is, after an interval of several years or decades. This suggests that these factors may influence the subsequent risk of myocardial infarction even after a considerable time, as previously indicated from data on the discontinuation of oral contraceptive use.[16]

The negative relationship between age at first pregnancy or birth and the risk of ischemic heart disease, if confirmed, would have important public health implications, particularly if a similar relationship is not restricted to younger and middle-aged women analyzed in the present and previous[8, 9] work but also applies to older age groups in whom there is a substantially greater incidence of coronary events.[5, 10] Therefore it appears that the age at which a woman bears her first child is an important correlate of the subsequent history of several important diseases in terms of either direct (i.e., increased risk of breast cancer with increasing age at first livebirth)[17] or, possibly, inverse association (i.e., decreased risk of ischemic heart disease with older age at first livebirth).

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The "breakfast tolerance test": Screening for gestational diabetes with a standardized mixed nutrient meal

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In a group of 50 presumed normal pregnant women and 20 known gestational diabetic women, all in the early third trimester, the function of a standard 50 gm, 1-hour screening test for gestational diabetes was compared with that of a plasma glucose level determined 1 hour after the ingestion of a standard 600 kcal mixed nutrient breakfast (breakfast tolerance test). The mean plus 2 SD for the breakfast tolerance test was 120 mg/dl. If this were used as the threshold for a screening test, 75% of cases of gestational diabetes would be identified (sensitivity), while 94% of normal pregnant women would be excluded (specificity). A threshold of 100 mg/dl yields a sensitivity of 96% and a specificity of 74%. These results are compared with those for the standard 50 gm glucose challenge. (AM J OBSTET GYNECOL 1987;157:1113-7.)

Key words: Gestational diabetes, diabetes screening test in pregnancy, glucose challenge in pregnancy, carbohydrate metabolism in pregnancy

The Second International Workshop Conference on Gestational Diabetes[1] has recommended that all gravid women be screened with a 50 gm, 1-hour glucose challenge at approximately 26 weeks of gestation, in order to detect the presence of gestational diabetes. The 50 gm screening test has been investigated extensively, and much is known about the manner in which this test functions.[2-4] However, logistic and economic disadvantages to using a carbohydrate load for screening make it advantageous to use some other type of screening test. Some investigators have explored the validity of a random glucose value, evaluated with[5] or without[6] reference to the interval since the last meal. Unfortunately, the content of the last meal was unknown in the former study,[5] and only 2.5% of the subjects had an oral glucose tolerance test, a number insufficient to determine the validity of the thresholds used. In addition, the criterion used in the above study for abnormality on the glucose tolerance test has not been validated for pregnancy. In the latter study,[6] only 48% of subjects with "impaired glucose tolerance in pregnancy" were detected by the random glucose screen. Nineteen percent had normal random values but underwent oral glucose tolerance tests because of historical risk factors and 33% did not have the random glucose screen. The authors recommended the random glucose determination as an adjunct to historical risk factors in choosing patients for glucose tolerance testing.

It is known that when carbohydrate is consumed with other nutrients, as in a mixed meal, the glycemic excursion is altered from that induced by an isocaloric pure carbohydrate load.[7] Thus it would be expected that the content of a recently ingested meal would influence the glucose level observed. The present investigation is designed to explore the function of a screening test for gestational diabetes based on the plasma glucose level observed 1 hour after the ingestion of a standardized mixed nutrient breakfast.

Subjects and methods

The subjects of this study include the same 70 pregnant individuals previously described in an investigation of the influence of fasting versus fed state on the 50 gm, 1-hour screening test.[4] Fifty were presumed normal women about to undergo screening for gestational diabetes, and an additional 20 had already been diagnosed as having gestational diabetes by the criteria of O'Sullivan and Mahan[8] as modified by Carpenter and Coustan.[3] Both groups of subjects were recruited from private obstetricians' offices and clinics, affiliated with the Women and Infants' Hospital of Rhode Island. Subjects were between 25 and 33 weeks of gestation at the time of the study; those with known gestational diabetes were approximately 3.5 weeks more advanced in gestation than presumed normal subjects, because they had already undergone the routine 50 gm, 1-hour

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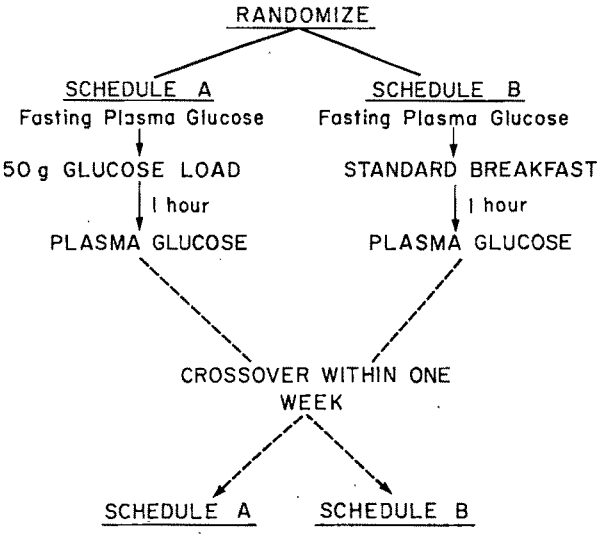


Fig. 1. Protocol for breakfast tolerance test.

screening test and the full 3-hour oral glucose tolerance test in the course of identification of the carbohydrate intolerance. Women with gestational diabetes were studied before starting on their dietary regimen. No patient was receiving insulin at the time of study. Demographic data describing these 70 individuals are presented in Table I.

The experimental protocol was approved by the Research and Human Subjects Committee of the Women and Infants Hospital of Rhode Island. All subjects fasted overnight before reporting for study. Twenty-four–hour dietary recall forms revealed that all subjects had ingested a minimum of 200 gm of carbohydrate on the day before the study. By means of a table of random numbers, the subjects were assigned one of two test schedules (Fig. 1). For schedule A, a sample for determination of the fasting plasma glucose level was first drawn, and subjects then drank a 50 gm oral glucose load (Limeondex, Curtin Matheson Scientific, Inc., Houston, Texas) over a period of 5 minutes. Plasma glucose was again measured 1 hour later. For schedule B, the subjects also had a sample drawn for determination of the fasting plasma glucose level but then consumed a standard mixed breakfast, consisting of approximately 600 kcal (Table II). One hour after completion of the mixed meal, a plasma glucose level was determined. As part of the previously published protocol,[4] the 50 gm glucose load was next administered, and a plasma glucose level was again obtained 1 hour later. Within 1 week, each subject was retested under the opposite conditions of the original test. The 50 presumed normal subjects and the 20 with gestational diabetes all completed the same protocol but were considered as two separate groups for purposes of randomization.

Blood samples were collected on ice in heparinized tubes and centrifuged within 1 hour. The plasma glucose level was determined by the glucose oxidase method (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Presumed normal subjects were evaluated with a 3-hour, 100 gm oral glucose tolerance test if either 50 gm screening test value exceeded or equaled 130 mg/dl. The criteria of O'Sullivan and Mahan,[8] as modified for plasma and glucose oxidase,[3] were used as the diagnostic standard for gestational diabetes. All subjects with known gestational diabetes had previously been diagnosed with such a test, and oral glucose tolerance tests were not repeated in this group.

Mean values were compared with the two-tailed *t* test.

Results

Among the 50 presumed normal subjects, 24 underwent a 100 gm, 3-hour oral glucose tolerance test because one or both of the 50 gm screening tests met or exceeded 130 mg/dl. Among these 24, four met the criteria for gestational diabetes. It should be noted that all subjects whose 1-hour postbreakfast plasma glucose level was ≥100 mg/dl underwent a 3-hour glucose tolerance test, as all had one or both 50 gm, 1-hour screening test values equal to or exceeding 130 mg/dl.

Among the 46 confirmed normal subjects, the mean (±SD) plasma glucose level 1 hour after the test meal was 85.6 ± 17 mg/dl (Table III). Thus the mean + 2 SD was 119.6 mg/dl. The postmeal glucose levels of two of the presumed normal subjects with gestational diabetes exceeded 120 mg/dl, but those of the other two did not (100 and 101 mg/dl). There were three confirmed normal subjects whose postmeal plasma glucose value exceeded this threshold, and all three had elevated 50 gm, 1-hour screening test values but normal 100 gm, 3-hour glucose tolerance tests.

Sixteen of the 20 subjects with known gestational diabetes exhibited a 1-hour postmeal plasma glucose level ≥120 mg/dl, while four did not (87, 106, 111, and 114 mg/dl). The 20 known gestational diabetic individuals were combined with the four cases of gestational diabetes diagnosed among the 50 presumed normal individuals in order to calculate sensitivity estimates. Thus, with a threshold of ≥120 mg/dl, the sensitivity of this test was 75% and the specificity 94%. As shown in Fig. 2, lowering the threshold to 100 mg/dl increases the sensitivity of the test to 96% but decreases the specificity to 74%.

In order to quantitate the degree of glucose intolerance in individual subjects, the sum of the amount by which the two most abnormal oral glucose tolerance test values exceeded the upper limits of normal[9] was calculated (Δ glucose tolerance test value). The mean Δ glucose tolerance test value for the 20 known ges-

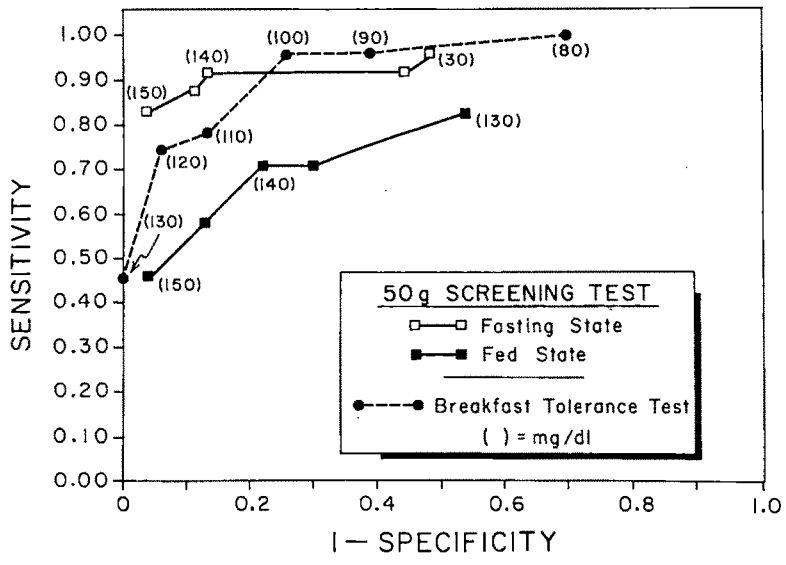


Fig. 2. Receiver operating characteristic curve for breakfast tolerance test and 50 gm, 1-hour screening test administered to fasting and fed subjects.

Table I. Demographic data

	Presumed normal subjects (N = 50)	*Known gestational diabetic women (N = 20)*
Age (yr) (mean ± SD)	27.4 ± 3.4	30.0 ± 5.0
Gestational age at meal study (wk) (mean ± SD)	26.9 ± 1.3	30.4 ± 2.2
Gestational age at no-meal study (wk) (mean ± SD)	27.0 ± 1.2	30.5 ± 2.0
Obesity*	4/50 (8%)	4/20 (20%)
Other risk factors†	18/50 (36%)	11/20 (55%)
No risk factors	29/50 (58%)	8/20 (40%)

*Greater than eighty-fifth percentile relative weight for height and age (National Center for Health Statistics, 1983).
†Family history of diabetes, previous macrosomic baby (>9 pounds), poor obstetric history.

tational diabetic patients in this study was 51.4 ± 47.0 mg/dl, and the median was 30 mg/dl. The six cases not detected by the postmeal plasma glucose determination at a threshold of 120 mg/dl had a mean Δ glucose tolerance test of 20.7 mg/dl (range 11 to 39) and thus, as a group, had mildly abnormal glucose tolerance test results.

Comment

In the present study, the plasma glucose level 1 hour after a standard mixed-nutrient breakfast was evaluated as a screening test for gestational diabetes. Because of patient sampling methods, positive and negative predictive accuracy cannot be evaluated. Sensitivity (the proportion of individuals with the disease who are correctly identified by the test) and specificity (the proportion of individuals free of the disease who are correctly excluded by the test) can be assessed, however, because these parameters are relatively unaffected by disease prevalence.

The receiver operating characteristic curve (Fig. 2) is a convenient way to express the function of a screening test.[10] In this curve, sensitivity is plotted against (1 − specificity), so that the reader can see at a glance how much sensitivity is gained with a given decrease in the test threshold and how much specificity is lost in the process. Test sensitivity increases from 75% at a threshold of ≥120 mg/dl to 96% at ≥100 mg/dl. As with other screening tests, the threshold is chosen to maximize test sensitivity while maintaining good test

Table II. Standard 600 kcal test breakfast

Menu	
Two scrambled eggs (3 oz)	
Two slices toast or one English muffin	
Two pats of butter or margarine	
8 oz orange juice	
8 oz whole or skim milk	
One cup coffee or tea (no sugar)	
Contents	
Carbohydrate	52 gm (210 kcal)
Protein	28 gm (110 kcal)
Fat	31 gm (280 kcal)

Table III. Plasma glucose level 1 hour after breakfast

	Normal subjects	*Gestational diabetic subjects*
Mean (mg/dl)	85.6	128.7
SD	16.9	21.8

df = 68; t = 8.22; $p < 0.00001$ (two-tailed *t* test).

specificity. The "breakpoint" of the receiver operating characteristic curve is that value at which the slope of increasing sensitivity falls, thus signifying a falloff in specificity without significant further gain in sensitivity.

The receiver operating characteristic curve for the breakfast tolerance test is compared with those for the 50 gm, 1-hour screening test administered in the fasting and fed states (Fig. 2). The "breakpoint" of the receiver operating characteristic curve for the breakfast tolerance test (100 mg/dl) is less discrete than that of the 50 gm, 1-hour screen in the fasting state (140 mg/dl) but more discrete than that of the 50 gm, 1-hour screen in the fed state (140 or 130 mg/dl). Thus the breakfast tolerance test functions slightly less efficiently than the 50 gm, 1-hour screen given to fasted subjects but more efficiently than the 50 gm, 1-hour screen in fed subjects.

Previous studies have suggested using a random plasma or whole-blood glucose determination as a screening test for gestational diabetes. Stangenberg et al.[6] (1985) described 6969 random capillary blood glucose measurements among 1500 pregnant women, reporting a mean of 83 mg/dl and a 95% upper confidence limit of 114 mg/dl. With a threshold of 116 mg/dl, 174 of the women had positive screening results on at least one occasion. Ten (0.7%) had abnormal carbohydrate metabolism by nonpregnancy glucose tolerance test standards. An additional four cases of gestational diabetes were diagnosed on the basis of a glucose tolerance test performed because of clinical suspicion, despite a "normal" random glucose measurement. Thus the sensitivity of this repetitive random capillary blood glucose screening test is, at best, 71%. It might be even lower, since 86.4% of the 1500 individuals had no test other than the random glucose determinations, and there is no information as to how many cases of gestational diabetes might have been missed by the screening test.

In the study of Lind and Anderson[5] (1984), 2403 patients had a venous plasma glucose determination between 28 and 32 weeks' gestation. On the basis of the patients' reports of the time of last meal ingestion, the results were divided into those from individuals who had eaten within 2 hours of sampling and those from individuals who has last eaten more than 2 hours before sampling. For those who had eaten within 2 hours, the mean plasma glucose level was 79 ± 13 mg/dl, and the 99% cutoff limit was 109 mg/dl. For those who had last eaten more than 2 hours before sampling, the mean was 73 ± 11 mg/dl and the 99% cutoff was 100 mg/dl. In 38 (1.4%) of the subjects the random glucose concentration exceeded (or nearly exceeded) the 99% cutoff, and a 75 gm oral glucose tolerance test was performed. Two of the 38 subjects had diabetes according to the World Health Organization criteria, and 11 had impaired glucose tolerance during pregnancy. Another 21 patients with normal random glucose screening tests underwent oral glucose tolerance testing for various clinical and historical indications. Three had impaired glucose tolerance. The low yield of gestational diabetes (0.25%) is about one tenth of that reported in most series that used other well-documented criteria and suggests that the morbidity associated with gestational diabetes would not be adequately predicted with this test. In fact, only 59 of the subjects (2.5%) had any testing other than the random capillary glucose determinations to look for gestational diabetes. Those authors assume that all of the 97.5% of patients not subjected to further testing would have had normal results had glucose tolerance tests been performed.

Recently, Mortensen et al.[11] investigated the use of the fasting whole-blood glucose level as a screening test for gestational diabetes in a population of 2448 pregnant women. A threshold of 73 mg/dl exceeded on two occasions prompted performance of a standard oral glucose tolerance test. Unfortunately, only patients with historical risk factors or glycosuria (7.8% of their population) were screened with fasting whole-blood glucose determinations. Although a control group of 54 nonscreened patients was tested with an oral glucose tolerance test at 28 weeks' gestation, the fact that none were diabetic does not imply that the incidence of gestational diabetes was negligible in the remaining 2394 untested patients, since the protocol described identified gestational diabetes in only 0.98% of the population.

The possible use of a fasting or random circulating glucose determination to detect gestational diabetes has not been tested in any substantial population, since screening tests must be validated by some confirmatory test (either the standard diagnostic test or some other standard screening test whose functions are already known) performed on the entire screened population. In the above three studies confirmatory testing was performed in only an extremely limited proportion of the screened population. The meal study reported herein was performed with the usual 50 gm, 1-hour screening test twice (once in the fed state and once in the fasting state) on all subjects screened. Since those subjects with 50 gm screening test values below 130 mg/dl on two occasions are extremely unlikely to have gestational di-

abetes,[3] the standard 3-hour, 100 gm oral glucose tolerance test was performed only in those patients with abnormal screens.

The values determined in the present study for plasma glucose 1 hour after a standard mixed-nutrient breakfast are similar to those in other reports in which this determination was not proposed as a screening test. Gillmer et al.[12] investigated glycemic excursions after a mixed breakfast containing 40 gm of carbohydrate (but an unreported amount of other nutrients) in 24 normal and 13 "chemical diabetic" women during the third trimester. They reported mean plasma glucose values of approximately 5 ± .5 mmol/L (89 ± 9 mg/dl) 1 hour after this meal among the normal individuals and approximately 6.2 ± 1.5 mmol/L (110 ± 27 mg/dl) among the "chemical diabetic" women. Cheney et al.[13] administered a 400 kcal breakfast (39 gm carbohydrate, 17 gm protein, 20 gm fat) to six normal, 23 lean gestational diabetic, and 12 obese gestational diabetic women at 35 weeks of pregnancy. The six normal women manifested a mean (±SE) plasma glucose level of 100 ± 5 mg/dl 1 hour after the breakfast. The lean gestational diabetic subjects in the study of Cheney et al. manifested a mean 1-hour plasma glucose level of approximately 125 ± 5 (SEM) mg/dl, similar to the 126 ± 21 (SD) mg/dl value obtained in our 19 nonobese gestational diabetic subjects. The obese gestational diabetic women in the study of Cheney et al. manifested a mean 1-hour plasma glucose level of approximately 150 ± 8 (SEM) mg/dl, somewhat higher than the 139 ± 24 (SD) mg/dl in our five obese gestational diabetic subjects.

The present study illustrates that the use of a standard mixed-nutrient meal as the provocative test for gestational diabetes screening may be as efficient as the use of a pure carbohydrate load, provided that the standard meal is one with a predictable effect on plasma glucose. This raises the possibility that some other standard oral challenge, which is palatable and prepackaged or easily measured and prepared, may be more readily administered, particularly in circumstances of limited financial resources.

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Gestational diabetes: Maternal response to diet and insulin therapy as reflected by glycosylated hemoglobin concentration

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To assess the effects of diet and insulin therapy on pregnancy complicated by gestational diabetes, glycosylated hemoglobin concentration was determined longitudinally in 32 women. Diet was instituted when a diagnosis of gestational diabetes was made and was supplemented with insulin for fasting hyperglycemia. At initial presentation, glycosylated hemoglobin concentration was increased in the 18 women who required insulin compared with the 14 women managed by diet alone (7.1% ± 0.2% versus 6.2% ± 0.2%, mean ± SEM, $p < 0.01$). Diet had no effect on glycosylated hemoglobin concentration that remained elevated to 6.1% ± 0.3% compared with the glycosylated hemoglobin concentration of 5.6% ± 0.2% for pregnant nondiabetic women ($p < 0.01$). Insulin resulted in a decrease in glycosylated hemoglobin concentration within 3 to 5 weeks ($p < 0.05$). After 7 to 9 weeks of insulin and diet, the glycosylated hemoglobin concentration in women with fasting hyperglycemia was the same as the glycosylated hemoglobin concentration in women who were managed by diet alone. (AM J OBSTET GYNECOL 1987;157:1118-21.)

Key words: Gestational diabetes, glycosylated hemoglobin concentration, diet and insulin therapy

Gestational diabetes, a frequent complication of pregnancy with an incidence of 1.6% to 3.0%, is associated with fetal macrosomia, as well as increased maternal morbidity and perinatal morbidity and death.[1, 2] In addition, there may be long-term sequelae of the abnormal maternal metabolism associated with gestational diabetes on both the mother and the developing fetus.[3-5]

The standard therapy for gestational diabetes is dietary counseling supplemented with insulin therapy if the woman maintains repetitive fasting hyperglycemia. Although dietary therapy has been demonstrated to decrease perinatal death, a perinatal morbidity rate of 25% persisted in gestational diabetic women who maintained fasting euglycemia.[6] O'Sullivan,[7] Oppermann and Camerini-Davalos,[8] and Coustan and Imarah[9] have demonstrated that prophylactic insulin treatment of gestational diabetic women significantly reduced the incidence of fetal macrosomia and the associated operative delivery and birth trauma. The subsequent development of overt diabetes by these gestational diabetic women later in life was also reduced by insulin therapy.[3]

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Estimates of diabetes control based on sporadic measurements of glucose in urine or blood do not provide the requisite precision for adequate assessment of the efficacy of dietary and insulin therapy. Glycosylated hemoglobin concentration has been demonstrated to reflect glycemic control during the past 4 to 8 weeks in both nonpregnant and pregnant diabetic women.[10, 11] Therefore the present study was designed to assess the effects of dietary and insulin therapy in pregnancy complicated by gestational diabetes by serial measurement of glycosylated hemoglobin concentration.

Methods

Thirty-two women who developed gestational diabetes were studied longitudinally throughout pregnancy between April 1983 and August 1985 at Duke University Medical Center. Informed consent was obtained from all study patients. Women in whom diabetes was present before pregnancy were excluded.

A 100 gm oral glucose tolerance test was performed if the plasma glucose concentration 1 hour after a 50 gm oral glucose load exceeded 134 mg/dl.[12] This screening test was performed routinely at the first prenatal visit and repeated (on nondiabetic women) after 26 weeks' gestation. Other indications for the oral glucose tolerance test included excessive weight gain, fetal macrosomia, and polyhydramnios. Gestational diabetes was diagnosed by the criteria of the National Diabetes Data Group.[13]

When gestational diabetes was diagnosed, a registered dietician provided dietary instruction based on the American Diabetes Association principles of nutri-

tion.[14] Daily caloric recommendations were approximately 35 kcal/kg ideal body weight. Repeat dietary counseling was provided if excessive weight gain, fetal macrosomia, or polyhydramnios developed.

Insulin therapy was begun if the fasting plasma glucose concentration exceeded 104 mg/dl two or more times within a 1- to 2-week interval. Insulin therapy consisted of a split/mixed regimen of regular and NPH Humulin insulin (Eli Lilly) with both morning and afternoon administration. The women receiving insulin therapy practiced home blood glucose monitoring (Ames Glucometer) at least two to four times daily to determine appropriate insulin adjustments.

Eighteen of the 32 gestational diabetic women required insulin therapy. These women who required insulin were similar in age and incidence of prior pregnancy loss and chronic hypertension when compared with the diet-controlled group (Table I). Gestational age at the time of enrollment into the study was the same for the two study groups. Although overall weight gain during gestation was similar for the two study groups, the incidence of obesity (greater than 20% above ideal body weight) was significantly higher for the women who received insulin therapy ($p < 0.02$). None of these 18 women required insulin therapy after the completion of their pregnancy.

Glycosylated hemoglobin concentration was determined at 2- to 4-week intervals after enrollment in the study and at the 4- to 6-week postpartum visit. Glycosylated hemoglobin concentration was measured by affinity chromatography with m-amino-phenylboronic acid as previously described.[15] Statistical comparisons were performed by use of the Student *t* test or Fisher's exact test.

Results

The glycosylated hemoglobin concentration at initial presentation of the women who required insulin for the management of fasting hyperglycemia was compared with the glycosylated hemoglobin concentration of the women who were managed by dietary therapy alone. The glycosylated hemoglobin concentration in the insulin-treated group was significantly increased over the group managed with dietary therapy alone, 7.1% ± 0.2% and 6.2% ± 0.2% (mean ± SEM), respectively ($p < 0.01$).

Four to 6 weeks postpartum, the glycosylated hemoglobin concentration was similarly increased in those women who required insulin therapy; it was 7.2% ± 0.4% and 6.1% ± 0.2% in the insulin and dietary treatment groups, respectively ($p < 0.01$).

The effects of therapy on glycosylated hemoglobin concentration in the women treated with dietary therapy alone are presented in Table II. The women in this group were enrolled at 20.1 ± 2.4 weeks' gestation, and dietary therapy instruction was performed at 23.1 ± 2.0 weeks. Throughout the remainder of gestation, there was no change in the glycosylated hemoglobin concentration, even after 11 to 13 weeks of conventional dietary therapy.

Table I. Clinical characteristics of the two study groups

Clinical characteristics	*Diet alone (n = 14)*	*Diet plus insulin (n = 18)*
Age (yr)	29.4 ± 0.9	31.9 ± 1.6
Prior pregnancy loss (%)	36	33
Chronic hypertension (%)	21	44
Weight gain (lb)	26.8 ± 3.7	26.1 ± 4.0
Obesity (%)	57	95*
Enrolled (wk)	20.1 ± 2.4	21.8 ± 1.5
Diet therapy (wk)	23.1 ± 2.0	22.1 ± 1.6
Insulin therapy (wk)	—	26.3 ± 1.6

Mean ± SEM.
*$p < 0.02$.

The effects of therapy on glycosylated hemoglobin concentration in women treated with dietary plus insulin therapy caused by repetitive fasting hyperglycemia are presented in Table III. The women in this group were enrolled at 21.8 ± 1.5 weeks' gestation. Dietary therapy instruction was performed at 22.1 ± 1.6 weeks, and insulin therapy was begun at 26.3 ± 1.6 weeks.

In this group requiring insulin therapy, the glycosylated hemoglobin concentration was not changed by dietary therapy alone. However, the glycosylated hemoglobin concentration of 7.3% ± 0.3% at initiation of insulin therapy significantly declined to 6.8% ± 0.2% after 3 to 5 weeks of insulin therapy ($p < 0.05$). The glycosylated hemoglobin concentration continued to decrease, and by 7 to 9 weeks of insulin therapy, the glycosylated hemoglobin concentration was down to 6.4% ± 0.3% ($p < 0.01$).

By the end of gestation, the glycosylated hemoglobin concentration of the diet alone and the diet plus insulin therapy groups was essentially the same, 6.1% ± 0.3% and 6.4% ± 0.3%, respectively. The incidence of a large for gestational age infant was also similar, 21% and 28% for the diet alone and diet plus insulin therapy groups, respectively. Likewise, the incidence of neonatal hyperbilirubinemia was similar for the two study groups, 30% and 27%, respectively. The neonatal plasma glucose concentrations at 1 hour of age were not significantly different at 39.1 ± 0.9 and 35.9 ± 2.2 mg/dl for the diet alone and diet plus insulin therapy groups, respectively.

Comment

The intent of conventional dietary and insulin therapy for gestational diabetes mellitus is the avoidance of maternal hyperglycemia. Maternal hyperglycemia is re-

Table II. Longitudinal analysis of glycosylated hemoglobin concentration of gestational diabetic women treated with diet therapy alone (n = 14)

*Glycosylated hemoglobin concentration (%)**				
	Dietary therapy (wk)			
Initial visit	*3-5*	*7-9*	*11-13*	*Postpartum*
6.2 ± 0.2	6.1 ± 0.3	6.3 ± 0.2	6.1 ± 0.3	6.1 ± 0.2

*Mean ± SEM.

Table III. Longitudinal analysis of glycosylated hemoglobin concentration of gestational diabetic women treated with combined dietary therapy plus insulin therapy (n = 18)

*Glycosylated hemoglobin concentration (%)**				
	Insulin therapy (wk)			
Initial visit	*0*	*3-5*	*7-9*	*Postpartum*
7.1 ± 0.2	7.3 ± 0.3	6.8 ± 0.2†	6.4 ± 0.3‡	7.2 ± 0.4

*Mean ± SEM.
†$p < 0.05$.
‡$p < 0.01$.

lated to fetal macrosomia.[2, 16] Fetal macrosomia is associated with perinatal complications such as maternal morbidity, birth trauma, neonatal hypoglycemia, neonatal hyperbilirubinemia, and perinatal mortality.

Prior studies have demonstrated that women who develop gestational diabetes mellitus consistently have elevated glycosylated hemoglobin concentrations throughout gestation compared with women who have normal carbohydrate metabolism.[15] Recent work has further demonstrated that maternal glycosylated hemoglobin concentration, which is measured by affinity chromatography, correlates closely with maternal plasma glucose concentration.[11]

Although most women with gestational diabetes may be able to avoid fasting hyperglycemia with dietary therapy alone, maternal nonfasting hyperglycemia persists as demonstrated by the inability of dietary therapy to normalize the maternal glycosylated hemoglobin concentration. Based on a longitudinal study of glycosylated hemoglobin concentration in nondiabetic women throughout gestation, the glycosylated hemoglobin concentration reaches a plateau of 5.6% ± 0.2% during the third trimester.[15] However, as demonstrated by this study, gestational diabetic women managed with dietary therapy alone maintained a glycosylated hemoglobin level elevated to 6.1% ± 0.3% ($p < 0.01$).

Therefore the results of this study suggest that conventional dietary therapy alone is of limited benefit in the control of nonfasting hyperglycemia in pregnancy complicated by gestational diabetes. This inability of conventional dietary therapy to normalize the glycosylated hemoglobin concentration is consistent with the demonstration by Gabbe et al.[6] that although dietary therapy may avoid fasting hyperglycemia and unexplained intrauterine fetal demise, there still is a 25% incidence of neonatal morbidity.

Conventional management of gestational diabetes mellitus recommends insulin therapy for repetitive fasting hyperglycemia after appropriate dietary therapy has been used. The present study demonstrated that the women who develop fasting hyperglycemia have a higher glycosylated hemoglobin concentration (7.1% ± 0.2%) in early gestation than gestational diabetic women who maintain fasting euglycemia (6.2% ± 0.2%, $p < 0.01$).

In addition, when insulin therapy was used because of repetitive maternal fasting hyperglycemia, the maternal glycosylated hemoglobin concentration decreased from 7.3% ± 0.3% to 6.4% ± 0.3% ($p < 0.01$). Therefore after only 7 to 9 weeks of combined insulin and dietary therapy, the glycosylated hemoglobin concentration in women with fasting hyperglycemia was the same as the glycosylated hemoglobin concentration in gestational diabetic women who had been managed by conventional dietary therapy alone. This finding is consistent with prior studies that have suggested that prophylactic insulin treatment of all gestational diabetic women reduces the incidence of fetal macrosomia and the associated operative delivery and birth trauma.[7-9]

In conclusion, the conventional therapeutic stan-

dards for gestational diabetes are inadequate for the prevention of nonfasting hyperglycemia and associated maternal and neonatal morbidity. This study provides the rationale for a randomized study to determine whether perinatal outcome can be improved by normalization of the glycosylated hemoglobin concentration throughout gestation by either more intensive dietary monitoring or combined insulin and dietary therapy.

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Preliminary observations on lactoferrin secretion in human vaginal mucus: Variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae*

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The concentration of the iron-binding protein lactoferrin was measured in vaginal mucus of women throughout the menstrual cycle. Lactoferrin is proposed to limit growth of mucosal pathogens such as *Neisseria gonorrhoeae*. Lactoferrin concentration in mucus was highest just after menses (62.9 to 218 μg/mg of protein) and lowest (3.8 to 11.4 μg/mg of protein) just before menses. Overall concentration of mucus protein showed no variation with menstrual cycle, and little lactoferrin was detectable in cell debris. Plasma lactoferrin did not show the variation seen in vaginal mucus. Because of the suggestion of hormonal influence on vaginal lactoferrin, its concentration was determined in women receiving oral contraceptive therapy. Mean lactoferrin concentration in women taking oral contraceptives was significantly lower than in the control group (never exceeding 19.8 μg/mg of protein) and showed no monthly variation. Vaginal lactoferrin appears to be under hormonal control. Variation in vaginal lactoferrin concentration may result in alterations in susceptibility to bacterial pathogens such as *Neisseriae gonorrhoeae*. (AM J OBSTET GYNECOL 1987;157:1122-5.)

Key words: Vaginal mucus, lactoferrin, *Neisseria gonorrhoeae*

Lactoferrin is a glycoprotein present in most secretions found at mucosal surfaces[1] and in secondary granules of polymorphonuclear neutrophils.[2] This protein may have a number of different functions,[3, 4] but its most striking feature is its high affinity for iron.[5] In this context, lactoferrin may play an important role in inflammation by removing free iron that otherwise might catalyze the formation of the hydroxyl radical[6] and by inhibiting growth of a variety of microorganisms that require iron for normal metabolism.[7] A direct bactericidal effect of lactoferrin has also been reported.[8]

The idea that lactoferrin might limit the growth of *Neisseria gonorrhoeae* has received particular attention because this pathogen is strictly limited to a human reservoir.[9] However, the relevance of this hypothesis is to a great extent dependent on the actual concentration of lactoferrin to which such organisms are exposed. In this article, we show that lactoferrin is found in remarkably high concentration in vaginal secretions with significant, reproducible variation during the menstrual cycle.

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Methods

Vaginal mucus collection. Eight healthy female volunteers were recruited. Half the subjects used combination oral contraceptives including Orthonovum 1/50 (Ortho Pharmaceutical, Rantam, N.J.) and Lo/Ovral (Wyeth Pharmaceutical). Specimens were collected according to a protocol approved by the Human Subjects Committee of the University of North Carolina. Subjects were instructed to place a 5 by 5 cm surgical sponge (Lisco, Kendal Company Hospital Products, Boston, Mass.) into their vagina on the morning of collection. Sponges had a nonabsorbable surgical suture attached to facilitate removal. Sponges were left in place for 24 hours, removed by the subjects, and placed immediately inside a sterile 10 ml plastic syringe (BD, Becton Dickinson and Co., Rutherfordton, N.J.). Specimens were returned within 1 hour of collection or kept at 4° C until return was possible. The sponge in the syringe was compressed to mobilize absorbed secretions and aliquoted directly (0.1 ml fractions) into Eppendorf tubes that were centrifuged at 15,000 g for 15 minutes.

Supernatant and pellet material was aliquoted and frozen at −70° C.

Timing of collections was as follows: Subjects waited 2 full days after their menstrual blood flow ceased, after which samples were obtained on 2 consecutive days. Subjects then waited 3 days and two additional samples were collected. This pattern continued until eight samples were obtained or menses ensued. Specimens from different subjects were grouped so that they were within 1 day of each other in the menstrual cycle, and results are reported in terms of the day most strongly represented by the samples available.

Preparation of plasma. Plasma from healthy volunteers was collected according to this schedule. Plasma was separated from whole blood as previously described.[10]

Determination of lactoferrin by enzyme-linked immunosorbent assay. Lactoferrin concentration was determined by a peroxidase-based enzyme-linked immunosorbent assay procedure as previously described.[10] Briefly, the immunoglobulin fraction of rabbit antihuman lactoferrin (Cappel No. 0201-1092) was diluted to 10 μg/ml in carbonate-coating buffer, pH 9.6. The 96-well microtiter plates (NUNC-Inter Med immunoplate 1 96F) were coated with 0.1 ml/well of this antibody overnight at 4° C. Each well was washed four times with 0.35 ml phosphate-buffered saline solution/Tween 20. Washings were done with a NUNC-Immuno Wash 12 (NUNC-Inter Med, Denmark) or automated microplate washer (Model EL402, Bio-Tek Instruments, Burlington, Vt.). Control testing showed similar results with either washing procedure. Purified human milk lactoferrin (Sigma L-8010, Sigma Chemical, St. Louis, Mo.) was diluted in 1% bovine serum albumin/phosphate-buffered saline and used for standards, with concentrations ranging from 1.6 to 64 ng/ml. The standards or unknowns were added to the plates, 0.1 ml/well, and incubated at 37° C for 1 hour. The plates were washed four times with phosphate-buffered saline solution/Tween 20.

Peroxidase-conjugated rabbit antihuman lactoferrin (Cappel No. 3201-1092) substrate diluted in 25 ml of citric acid buffer, pH 5.0, 25 ml of distilled H_2O, 20 mg/ml of phenylene diamine (Sigma P3888), and 0.018 ml of 30% H_2O_2, was added to the wells. Additions were done in 5-second intervals with a total reaction time of 4 minutes. The reaction was stopped with 8N H_2SO_4 and immediately read on a Bio-Tek microplate autoreader (Model No. EL309) at 492 nm. To ensure that the assay did not measure transferrin, control experiments were conducted in which human transferrin (Sigma) was added to the enzyme-linked immunosorbent assay. Interference was noted only at milligram concentrations, whereas transferrin was not detected in vaginal mucus by rate nephelometry sensitive to 25 μg/ml.

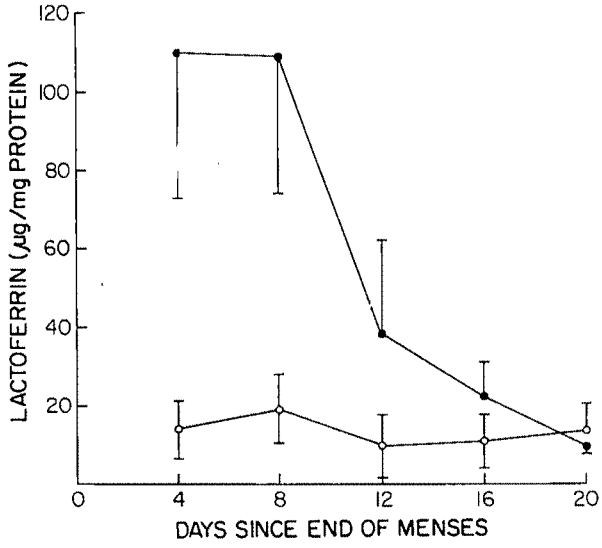


Fig. 1. Lactoferrin in the supernatant fluid of vaginal mucus (see Methods section) expressed per milligram of protein in the sample. Results are the mean of three separate donors in each group for whom adequate material was available for complete analysis in duplicate on two separate occasions. (o — o = Oral contraceptive pills; ● — ● = control.)

Results

Fig. 1 shows the mean concentration of lactoferrin in vaginal mucus in normal volunteers throughout their menstrual cycle. In women not receiving oral contraceptives, lactoferrin concentration was highest after menses, ranging from 62.9 to 218.0 μg/mg of protein, and was lowest just before the onset of menses, ranging from 3.87 to 11.4 μg/mg of protein. Because vaginal mucus protein was used as a denominator, we measured its variations throughout the menstrual cycle. As shown in Fig. 2, protein concentration did not vary widely during the menstrual cycle. We also examined the lactoferrin concentration of material (cell debris) pelleted out during the collection of vaginal mucus (see Methods section). This material was richer in protein (4 to 8 mg/ml of secretion), but the lactoferrin concentration did not exceed 7.3 μg/ml in any sample.

The source of lactoferrin in vaginal mucus is not known. It is possible that leakage from serum may play a role. As shown in Fig. 3, plasma lactoferrin ranged from 0.355 to 0.769 μg/ml during the menstrual cycle. Plasma lactoferrin concentration was highest just before or during menses, but the large variation seen in vaginal secretions was not observed.

Our results suggested that variation in vaginal lactoferrin was related to hormonal changes occurring

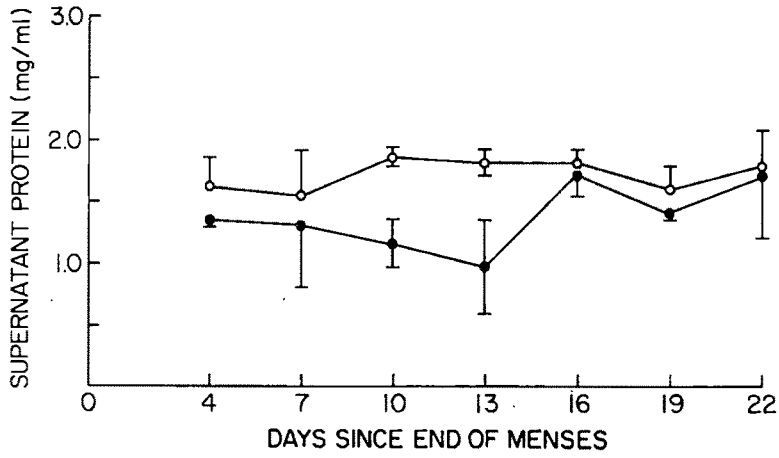


Fig. 2. Protein concentration in vaginal mucus during the menstrual cycle. Samples were examined as described in Fig. 1. (o—o = Oral contraceptive pills; •—• = control.)

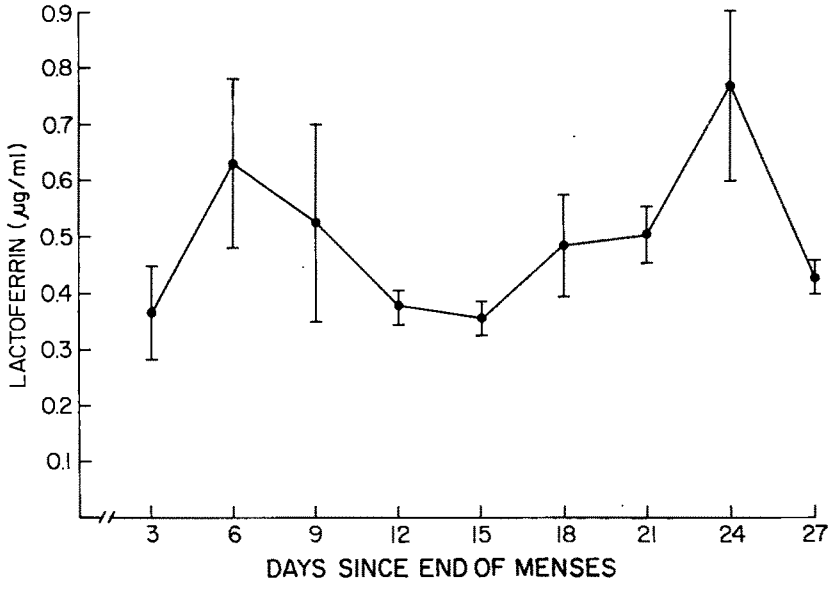


Fig. 3. The mean lactoferrin concentration in the plasma of three healthy volunteers. Samples were collected throughout the menstrual cycle and measured in duplicate.

during the menstrual cycle. To assess this hypothesis, we examined vaginal mucus secretion from women using oral contraceptives. As shown in Figs. 1 and 2, the mean lactoferrin concentration in women receiving oral contraceptive therapy was considerably lower than in the control group and did not exceed 19.8 µg/mg of mucus protein. The difference in lactoferrin secretion between control donors and donors receiving oral contraceptives was observed during most of the menstrual cycle (Fig. 1). Even more striking, lactoferrin concentration did not show monthly variation in women receiving oral contraceptive therapy (Fig. 1).

Comment

In this study we characterized lactoferrin secretion in the vaginal mucus of healthy volunteers. Lactoferrin was found predominantly in supernatant preparations free of cellular debris. Mean vaginal lactoferrin concentration in donors not receiving oral contraceptives ranged from 9 to 109 µg/mg of vaginal mucus protein, depending on the time of collection relative to menses. These concentrations of lactoferrin are consistent with an earlier report by Masson et al.,[1] in which lactoferrin was quantitated in "genital mucus" and was said to be between 0.5 and 1.0 mg/ml. This concentration of lactoferrin is greater than that found in most other secretions previously examined.[1]

Assays for detection of lactoferrin have received much attention.[10-12] Our assay was sensitive to nanogram concentrations of this protein and our results proved highly reproducible.[10] The concentrations of lactoferrin we detected in plasma were consistent with

the existing literature,[10-12] which further validated our assay. Because of the complex nature of vaginal mucus, we also performed "spiking" experiments in which known amounts of milk lactoferrin were added to vaginal mucus. Recovery in these experiments was 84% (n = 10, data not shown), so it seems likely that the lactoferrin values reported were accurate.

Lactoferrin concentration in vaginal mucus varied dramatically during the menstrual cycle and was lowest just before menses. Lactoferrin concentration was significantly reduced in women receiving oral contraceptives and failed to vary during the menstrual cycle. Earlier work has suggested hormonal effects on lactoferrin formation in other tissues: (1) Milk lactoferrin concentration decreases 4 days postpartum[13]; (2) neutrophil and serum lactoferrin are lower in women than men[11, 12]; (3) lactoferrin concentration is greater in the plasma of postmenopausal than premenopausal women[11]; and (4) lactoferrin staining in the endometrium is greater in the (later) secretory phase of the menstrual cycle.[14, 15]

The potential sources of lactoferrin in vaginal secretions include plasma (serum), neutrophils, and secretion by cervical or endometrial cells. The relative contribution of these sources remains unknown but given the concentration detected in vaginal mucus, it seems unlikely that plasma[10, 11] or polymorphonuclear neutrophils,[12] are of primary importance. The paradoxic increase in endometrial lactoferrin previously reported[14, 15] at a time when vaginal mucus lactoferrin is falling suggests that vaginal lactoferrin is derived from another source (e.g., endocervix[16]) that greater secretion from this tissue occurs early in the menstrual cycle, or that endometrial lactoferrin lost (shed) during menses remains in vaginal secretions after menses.

Given the amount of epidemiologic data used to explain the biology of gonococcal infection,[9] it seems warranted to speculate on how our findings might relate. Many organisms that produce disseminated gonococcal disease cannot use lactoferrin as an iron source.[9] Disseminated gonococcal infection frequently begins during menses.[9] At this time iron from other sources may reach the concentration required for the growth of these variants, overcoming the limitation imposed by lactoferrin. The effects of oral contraceptives (and concommitant lactoferrin suppression) on the biology of the vaginal flora remain to be determined.

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Human chorionic gonadotropin, estriol, and testosterone changes in two pregnancies with hyperreactio luteinalis

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A case of hyperreactio luteinalis is presented with measurements of human chorionic gonadotropin, total and free testosterone, and estriol throughout two term pregnancies. Ovulation induction with menotropins resulted in one spontaneous first-trimester abortion, one singleton term pregnancy, and 1 triplet pregnancy that converted to a singleton term pregnancy after spontaneous degeneration of two fetuses in the first trimester. During the first term pregnancy, levels of human chorionic gonadotropin peaked at 34 weeks of gestation (169,000 mIU/ml) and levels of total testosterone peaked at the same time (1777 ng/dl). Free testosterone (16.6 ng/dl) and estriol (10.8 ng/ml) reached their peak at 36 weeks of gestation. During the second term pregnancy, peak values of human chorionic gonadotropin (150,900 mIU/ml) and total testosterone (870 ng/dl) occurred at 10 and 5 weeks of gestation, respectively, before two fetuses of a triplet pregnancy degenerated. (AM J OBSTET GYNECOL 1987;157:1126-8.)

Key words: Hyperreactio luteinalis, testosterone, human chorionic gonadotropin, estriol, polycystic ovarian disease

Virilization during pregnancy may affect the mother alone or both mother and female fetus. Fetal virilization has been observed with androgen-producing tumors or with luteomas of pregnancy, but not with hyperreactio luteinalis. Maternal virilization occurs in 25% of cases with hyperreactio luteinalis and in 10% to 50% of all cases with luteoma of pregnancy.[1]

Reviews in the literature give only scant data on the underlying endocrine pathophysiology of hyperreactio luteinalis and luteoma of pregnancy. We had the opportunity to study a patient prospectively and longitudinally for several years from the time of diagnosis and treatment of anovulation and infertility to the completion of two term pregnancies. Observations of hormone measurements during this time are reported.

Case report

At the time of referral to the Reproductive Endocrine Unit, the patient was a 22-year-old Caucasian woman. She had normal breast and pubic hair development at 12 years of age. She subsequently experienced menarche at 13 years of age with irregular menstrual cycles every 1 to 3 months lasting 5 days. At 15 years of age, she was thin and muscular with facial acne and pigmented hair on her chin, upper lip, and cheeks. Laboratory data at that time revealed the following values: follicle-stimulating hormone (FSH), 7.6 mIU/ml (normal 5 to 25 mIU/ml); luteinizing hormone (LH), 28 mIU/ml (normal 4 to 30 mIU/ml); 17-ketosteroids, 10.6 mg/24 hr (normal 7 to 15 mg/24 hr); 17-hydroxycorticosteroids, 5.2 mg/24 hr (normal 2 to 11 mg/24 hr); and pregnanetriol, 3 mg/24 hr (normal 0 to 4 mg/24 hr). At the age of 22 years weight was 126 pounds, and height 5 feet 3 inches. The facial skin was oily with mild acne. Coarse, pigmented hair was present on chin, upper lip, cheeks, neck, chest, abdomen, and all extremities. Her voice had a low pitch. A slight recession of the temporal hair line was noted. On pelvic examination the glans of the clitoris measured 0.5 by 0.7 cm. Both ovaries were symmetrically enlarged to two to three times normal size. All other organs were normal as assessed by inspection and palpation. Ultrasound examination of the pelvis revealed enlarged, multicystic ovaries with no evidence of a tumor. Laboratory results showed levels of prolactin of 30 ng/ml (normal 0 to 35 ng/ml) and of serum testosterone of 188 ng/dl (normal 22 to 103 ng/dl). She was admitted to the Clinical Research Center for ovarian and adrenal stimulation and suppression and retrograde catheterization of the ovarian and adrenal veins combined with hormone analysis. Androgen levels of ovarian vein blood excluded the presence of an androgen-producing tumor: In the left ovarian vein the level of testosterone was 118 ng/dl and that of dihydrotestosterone was 67 ng/dl. In the right ovarian vein the testosterone level was 129 ng/dl and the dihydrotestosterone level was 97 ng/dl. Suppression of adrenocortical function with 2 mg of dexamethasone for 5 days resulted in a marked decrease of 17-hydroxycorticosteroids excretion in the urine and no change in urinary 17-ketosteroids, serum testosterone, dihydrotestosterone, estrone, and estradiol excretion. Ovarian stimulation with intramuscular injections of 5000 U human chorionic gonadotropin (hCG) for

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Table I. Blood levels of hCG, testosterone, and estriol during first term pregnancy (singleton)

Gestational weeks	*hCG (mIU/ml)*	*Total testosterone (ng/dl)*	*% Free testosterone*	*Free testosterone (ng/dl)*	*Estriol (ng/ml)*
10	60,000	358	0.91	3.3	ND
15	58,000	347	0.68	2.4	<1
19	67,000	511	0.82	4.2	<1
22	59,000	546	0.75	4.1	2.5
26	50	855	0.86	7.4	ND
30	71,000	1,344	0.92	12.4	3.9
34	169,000	1,777	0.88	15.6	5.5
36	132,000	1,677	0.99	16.6	10.8
40	66,000	881	1.14	10	—
Post partum, 2 wk	—	80	—	—	—
Umbilical cord blood	120	282	4.16	11.7	148

ND, Not detectable. Normal values are as follows: hCG, 40 to 300,000 mIU/ml; total testosterone, 68 to 360 ng/dl; % free testosterone, 0.23 to 0.7; free testosterone, 0.9 to 3.2 ng/dl; estriol, 0 to 17.7 ng/ml.

Table II. Blood levels of hCG, testosterone, and estriol during second term pregnancy (triplet pregnancy converted to singleton)

Gestational weeks	*hCG (mIU/ml) (normal 40-300,000)*	*Total testosterone (ng/dl) (normal 68-360)*	*% Free testosterone (normal 0.23-0.7)*	*Free testosterone (ng/dl) (normal 0.9-3.2)*	*Estriol (ng/ml) (normal 0-17.7)*
5	644	870	0.24	19.3	0.1
10	150,900	821	0.24	19.7	0.2
15	140,000	271	0.51	13.9	0.2
19	47,300	335	0.35	11.8	1.7
22	48,700	311	0.41	12.8	1.8
26	41,500	374	0.39	14.5	3.9
30	41,000	521	0.32	16.5	4.2
34	54,200	480	0.53	25.2	4.7
36	72,000	654	0.41	26.5	11.0
3 days post partum	91,600	292	0.64	18.7	ND*
Umbilical cord blood	61	726	0.48	35	154.5

*ND = Not detectable.

3 days in the presence of adrenocortical suppression with dexamethasone increased levels of testosterone from 93 to 220 ng/dl and of estradiol from 108 to 248 pg/ml without changing levels of dihydrotestosterone and estrone in peripheral blood. The rapid intravenous bolus injection of adrenocorticotropic hormone (0.25 mg cosyntropin) had no effect on peripheral serum levels of testosterone, dihydrotestosterone, estradiol, and estrone for 180 minutes. Serum estrone and estradiol levels were measured by radioimmunoassay after column fractionation. Estriol was determined by the Quantimune estriol radioimmunoassay (Biorad, Richmond, California). Total testosterone was determined by the Testosterone Test Set (Radioassay Systems Incorporated, Carson, California). Free testosterone was measured by the Coat-a-count Free Testosterone Radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California). Testosterone and dihydrotestosterone were separated by column chromatography and analyzed by radioimmunoassay. Human chorionic gonadotropin was measured by the Tandem-R hCG, Immuno Radio Metric Assay (Hybritech Incorporated, San Diego, California). All other hormone determinations were carried out by standard measurements in the Clinical Chemistry Laboratory of Strong Memorial Hospital, Rochester, N.Y.

On the basis of the clinical findings and laboratory values of an LH:FSH ratio of 3.7:1 combined with high levels of serum and urinary androgens, a diagnosis of polycystic ovarian disease was made. Treatment with clomiphene citrate in increasing doses did not result in ovulation. The addition of prednisone to therapy was equally unsuccessful in stimulating ovulation. The patient then underwent ovulation induction with human menopausal gonadotropin–hCG for five treatment cycles. This resulted in a first-trimester miscarriage after the third treatment cycle and a viable, singleton pregnancy after the fifth cycle. During the first term pregnancy the patient's voice deepened slightly and an increase in skin oiliness was noted. Total and free testosterone, hCG, and estriol levels were measured in intervals throughout pregnancy, after delivery, and in umbilical cord blood (Table I). At 41 weeks of gestation labor was induced because of hypertension, and she was delivered of a normal male infant by cesarean section with a birth weight of 4280 gm. The placenta was

normal in weight and appearance. The ovaries were markedly enlarged, smooth, and multicystic, measuring 15 by 10 by 8 cm bilaterally. The appearance of the ovaries was consistent with the diagnosis of hyperreactio luteinalis. The infant required operation because of pyloric stenosis at 3 months of age and has developed normally until this time. One year after delivery the patient desired pregnancy again. Laparoscopy revealed symmetrically enlarged, polycystic ovaries. On microscopic examination an ovarian biopsy specimen was consistent with a diagnosis of polycystic ovarian disease.

An interval of 2½ years passed between delivery of the first term pregnancy and conception of the second term pregnancy we describe. After two treatment cycles of human menopausal gonodotropin–hCG therapy, she conceived a triplet pregnancy. During the first trimester of the triplet pregnancy we observed several episodes of vaginal spotting. Ultrasound examination at 11 and 13 weeks of gestation documented that these bleeding episodes coincided with the degeneration and disappearance of two fetuses. A single male fetus was carried to term and delivered by repeat cesarean section (birth weight 4000 gm). The ovaries were similar in appearance and size as during the first cesarean section. The results of hormone measurements for this last pregnancy are summarized in Table II.

Comment

This case report of hyperreactio luteinalis in a patient with polycystic ovarian disease describes hormone regulation before pregnancy and during pregnancy. The predisposition of polycystic ovarian disease to the development of hyperreactio luteinalis or luteoma of pregnancy is established by other case reports in the literature. In our case, as well as in others with luteoma or hyperreactio luteinalis, the abnormal condition of maternal virilization was repetitive in two consecutive pregnancies. Selective adrenal and ovarian vein catheterization with androgen and estrogen determinations in the venous effluent was consistent with values reported in ovarian vein blood of hirsute women or women with polycystic ovaries. Stimulation and suppression tests of ovarian and adrenal function were performed in our patient before conception.

Androgen, estrogen, and hCG determinations during pregnancy and in umbilical cord blood are the most important data of our report. From 10 to 22 gestational weeks the hCG values remained relatively constant during the first term pregnancy. This was followed by extremely low measurements of 50 mIU/ml at 26 weeks and a peak of 169,000 mIU/ml at 34 weeks of gestation. During her second term pregnancy, peak levels of hCG were measured at 10 and 15 weeks of gestation. This difference between both pregnancies may be explained by the fact that the large placental mass of her second pregnancy associated with triplets produced more hCG during the first trimester. After the degeneration of two triplets, the functional placental mass decreased to that of a continuing normal singleton pregnancy. No comparable results can be found in the literature, as only single hCG determinations at term have been reported in cases of hyperluteinization.

The observations for total testosterone differ for both term pregnancies in our patient. While maximal values of 13 to 17 times baseline were measured in the third trimester of her first term pregnancy, the maximal increase in total testosterone was noted at 5 to 10 weeks of gestation of her second term pregnancy. Both peaks of total testosterone secretion coincide with the peaks of hCG secretion. Similar observations of increases in total testosterone with hyperluteinization have been reported by several authors. However, free testosterone data are available in only one case report.[2] Measurements of total testosterone in cord blood were 2.8 and 7.26 ng/ml in both term pregnancies of our patient. This compares with values of 0.26 to 4.65 mg/ml reported by others. Free testosterone levels in cord blood have not been reported. It is therefore difficult to put our values of 0.12 and 0.4 ng/ml of free testosterone in cord blood into perspective. Our observations for serum estriol in both term pregnancies fall within the normal range and show only minor differences between the first and second term pregnancies. The variations between both pregnancies may be explained by secretory differences related to placental mass. In our patient maternal virilization is explained by the increase of total and free testosterone in the maternal circulation. Fetal virilization cannot be assessed in our patient because both pregnancies resulted in male infants. The measurements of free and total testosterone in cord blood of both pregnancies suggest that fetal virilization in female offspring is possible with hyperreactio luteinalis.

REFERENCES

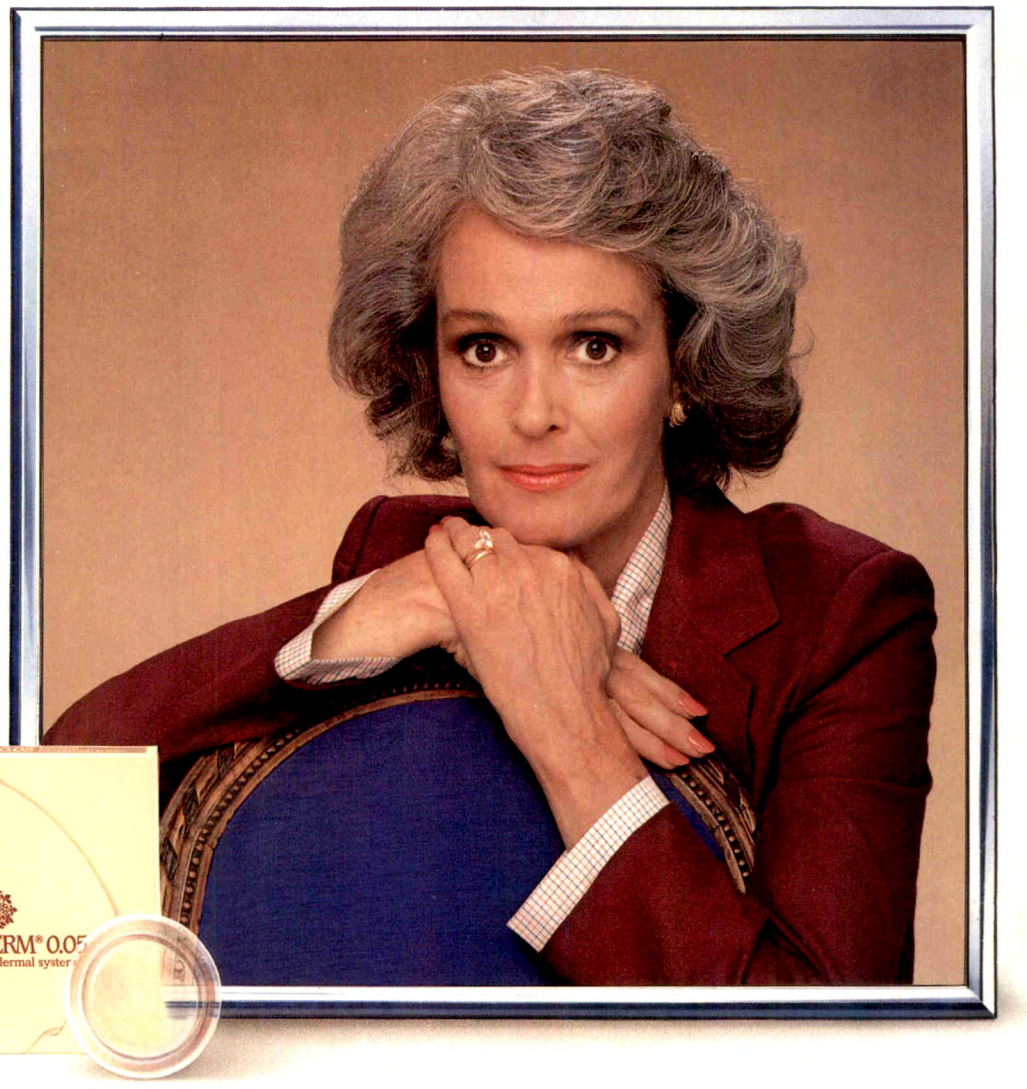
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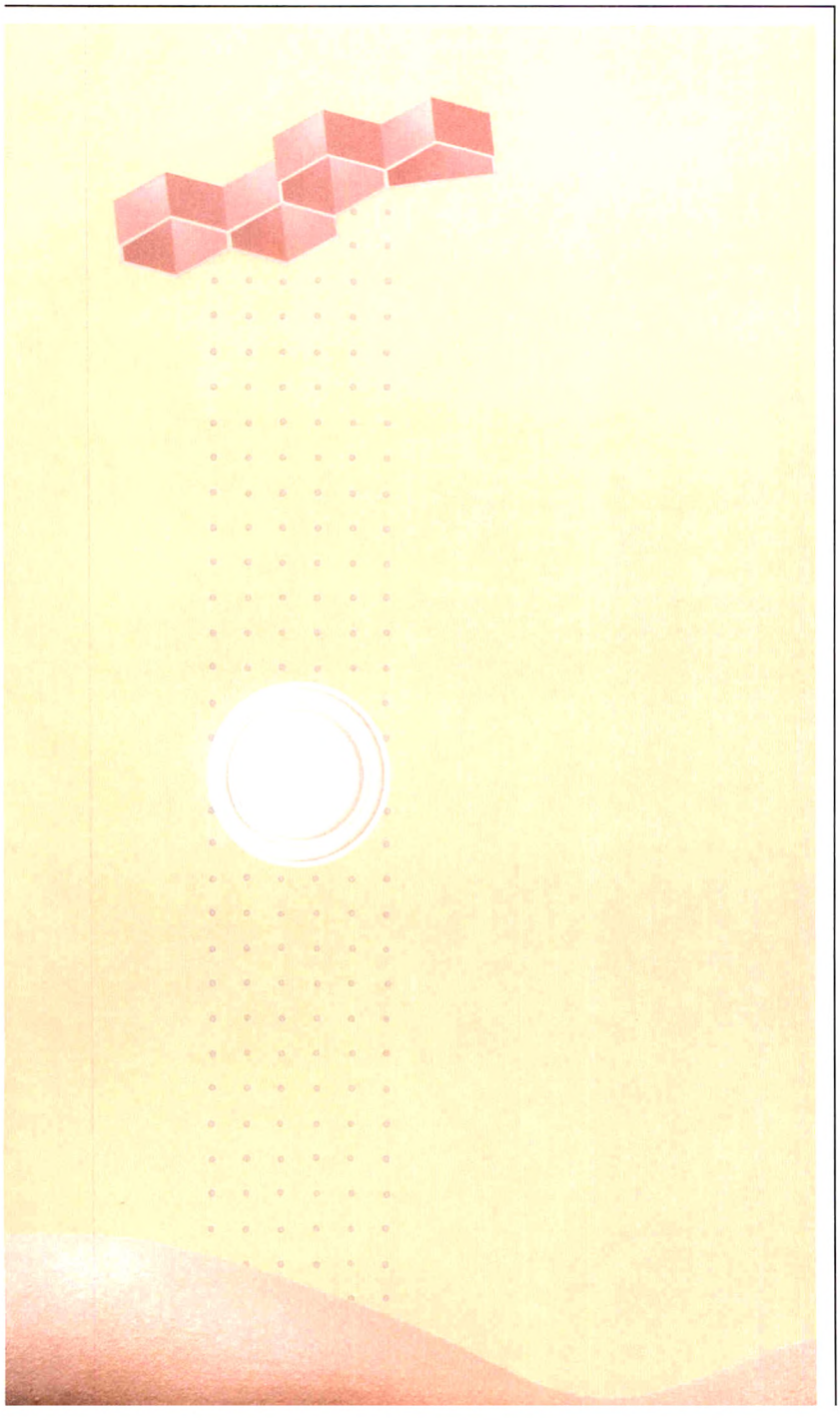
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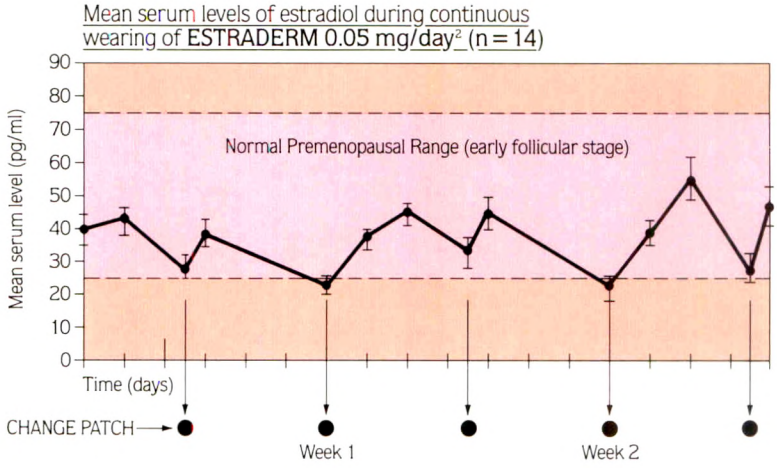
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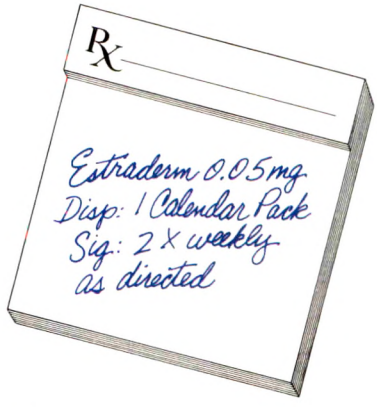
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References:

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BRIEF SUMMARY OF PRESCRIBING INFORMATION.
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ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than 1 year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer-reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5-13.9 times greater than in nonusers. The risk appears to depend both on duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that women who had been exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than four per 1000 exposures. Furthermore, a high percentage of such exposed women (30-90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data on the use of other estrogens are not available, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7-fold increased risk of limb-reduction defects in infants who had been exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estraderm is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and of the advisability of continuation of the pregnancy.

INDICATIONS AND USAGE

Estraderm is indicated for the treatment of the following: moderate-to-severe vasomotor symptoms associated with menopause; female hypogonadism; female castration; primary ovarian failure; and atrophic conditions caused by deficient endogenous estrogen production, such as atrophic vaginitis and kraurosis vulvae.

Studies of the addition of a progestin for seven or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of endometrium suggest that 12 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks that may be associated with the inclusion of progestin in estrogen replacement regimens. The potential risks include adverse effects on carbohydrate and lipid metabolism. The choice of progestin and dosage may be important in minimizing these adverse effects.

CONTRAINDICATIONS

Estrogens should not be used in women or men with any of the following conditions:

1. known or suspected cancer of the breast;
2. known or suspected estrogen-dependent neoplasia;
3. known or suspected pregnancy (see Boxed Warning);
4. undiagnosed abnormal genital bleeding;
5. active thrombophlebitis or thromboembolic disorders;
6. history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. Induction of Malignant Neoplasms. Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There are now reports that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time, there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of breast cancer, although a recent long-term follow-up of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. Gallbladder Disease. A recent study has reported a two- to threefold increase in the risk of surgically confirmed gallbladder disease in postmenopausal women receiving oral estrogens, similar to the twofold increase previously noted in users of oral contraceptives.

3. Effects Similar to Those Caused by Estrogen-Progestogen Oral Contraceptives. There are several serious adverse effects of oral contraceptives and other high-dose oral estrogen treatments, most of which have not, up to now, been documented as consequences of postmenopausal estrogen replacement therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women.

a. Thromboembolic Disease. It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least four weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found, this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. Hepatic Adenoma. Benign hepatic adenomas have been associated with the use of oral contraceptives. Although benign and rare, these tumors may rupture and cause death from intra-abdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations, but they should be considered if abdominal pain and tenderness, abdominal mass, or hypovolemic shock occurs in patients receiving estrogen. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The causal relationship of this malignancy to these drugs is not known.

c. Elevated Blood Pressure. Women using oral contraceptives sometimes experience increased blood pressure which, in most cases, returns to normal upon discontinuing the drug. There is now a report that this may occur with use of oral estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used. Ethinyl estradiol and conjugated estrogens have been shown to increase renin substrate. In contrast to these oral estrogens, transdermally administered estradiol does not affect renin substrate.

d. Glucose Tolerance. A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. Hypercalcemia. Administration of high doses of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If hypercalcemia occurs, use of the drug should be stopped and appropriate measures should be taken to reduce the serum calcium level.

PRECAUTIONS

General

1. A complete medical and family history should be taken before initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, as well as a cervical Papanicolaou test. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.
2. Because estrogens may cause some degree of fluid retention, careful observation is required when conditions that might be influenced by this factor are present (e.g., asthma, epilepsy, migraine, and cardiac or renal dysfunction).
3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as uterine bleeding, mastodynia, etc.
4. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Estrogens should be used with caution in patients who have or have had endometriosis.
5. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.
6. Preexisting uterine leiomyomata may increase in size during prolonged estrogen use. If this occurs, estrogen therapy should be discontinued while the cause is investigated.
7. In patients with a history of jaundice during pregnancy, there is an increased risk that jaundice will recur with the use of estrogen-containing oral contraceptives. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.
8. Estrogens may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients.
9. Because the prolonged use of estrogens influences the metabolism of calcium and phosphorus, estrogens should be used with caution in patients with metabolic bone diseases associated with hypercalcemia and in patients with renal insufficiency.

Drug/Laboratory Test Interactions

The results of certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following changes have been observed with large doses of oral estrogen:

1. increased sulfobromophthalein retention;
2. increased prothrombin time; increased factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability;
3. increased thyroxine-binding globulin (TBG), leading to increased circulating total thyroid hormone (T_4) as measured by column or radioimmunoassay; free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered; TBG was not affected in clinical trials of Estraderm;
4. reduced response to the metyrapone test;
5. reduced serum folate concentration;
6. increased serum triglyceride and phospholipid concentration, and decreased pregnanediol excretion.

The pathologist should be informed that the patient is receiving estrogen therapy when relevant specimens are submitted.

Carcinogenesis, Mutagenesis, Impairment of Fertility

See **WARNINGS** and Boxed Warning.

Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

Pregnancy Category X

See **CONTRAINDICATIONS** and Boxed Warning.

Estrogens should not be used during pregnancy.

Nursing Mothers

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

See **WARNINGS** and Boxed Warning regarding potential adverse effects on the fetus, induction of malignant neoplasms, increased incidence of gallbladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.

The most commonly reported adverse reaction to Estraderm in clinical trials was redness and irritation at the application site. This occurred in about 17% of the women treated and caused approximately 2% to discontinue therapy. Reports of rash have been rare.

The following additional adverse reactions have been reported with estrogenic therapy, including oral contraceptives:

Genitourinary System: Breakthrough bleeding, spotting, change in menstrual flow; increase in size of uterine fibromyomata; change in cervical erosion and amount of cervical secretion.

Endocrine: Breast tenderness, breast enlargement.

Gastrointestinal: Nausea, vomiting; abdominal cramps, bloating; cholestatic jaundice have been observed with oral estrogen therapy.

Eyes: Steepening of corneal curvature; intolerance to contact lenses.

Central Nervous System: Headache, migraine, dizziness.

Miscellaneous: Change in weight, edema, change in libido.

Do not store above 86°F (30°C).

Do not store unpouched. Apply immediately upon removal from the protective pouch.

CAUTION: Federal law prohibits dispensing without prescription.

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*Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These data represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to all data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

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BRIEF SUMMARY

Tri-Levlen™—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

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430-34

Carbon dioxide laser laparoscopy performed with a flexible fiber in humans

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A flexible carbon dioxide laser fiber delivery system was used in conjunction with the laparoscope to treat 12 women. The 77 by 2 mm hollow fiber could subtend an arc of 90 degrees without significant loss of power or unfavorably affecting the delivery mode. As many as 20 W of power was transmitted through the fiber; however, carbon dioxide gas flow rates as high as 2000 cc/min reduced power by approximately 25%. Replaceable tips preserved fiber integrity to the extent that a single fiber could be reused repeatedly and gas sterilized without jeopardizing laser transmission. Of the 12 cases, endometriotic implants were vaporized in eight women, adhesiolysis was performed in four women, endometrial cysts were drained in two women, and vaporization of myomata was accomplished in two patients. The fiber was also used as a manipulating instrument and could be internally bent with a grasping forceps to better site the laser beam on a lesion. No complications related to the use of the carbon dioxide laser fiber were observed. The safety aspects of carbon dioxide laser fiber technology are not inconsiderable and could provide laser laparoscopy with an extra margin of security. (Am J Obstet Gynecol 1987;157:1129-33.)

Key words: Carbon dioxide laser fiber, laser laparoscopy with carbon dioxide fiber, flexible carbon dioxide laser fiber

The carbon dioxide laser is the most useful and widely used system for gynecologic laser surgery because of its excellent control and superior vaporizing capabilities. The major drawback in using the carbon dioxide laser through endoscopes has been the lack of a small-caliber, flexible delivery system. Until recently the search for a carbon dioxide laser fiber had been elusive.

The first report of a practical and functional carbon dioxide fiber was published by Baggish and Elbakry[1] in 1986. This flexible fiber was used successfully to perform operative laparoscopy on rabbits and deliver the carbon dioxide laser beam with an acceptable mode and power retention. Although laser laparoscopy has proved to be an increasingly accepted, least invasive technique for the treatment of many pathologic conditions within the abdominal cavity, its major deficiency focuses on a cumbersome delivery system.[2-4] The single-puncture operating laparoscope delivers the laser beam through a 5 to 6 mm diameter operating channel and provides a relatively easy way to perform operative laser endoscopy. The problems associated with this technique relate to the focus of the laser beam being asynchronous to that of the laparoscope and the fact that the operator cannot accurately determine what structure(s) may be interposed between the laser beam and the target. Recent reports of injuries that involved intestinal trauma during laser laparoscopy with single-puncture methodology reinforce this concern. Use of a hollow laser probe through a second or third puncture allows the operator to manipulate the laser beam independent of the endoscope and to always know what lies between the laser probe and the target. Unfortunately, these rigid metal tubes measure 7 to 8 mm in diameter and require large trocars.

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Material and method

The carbon dioxide laser flexible fiber was provided and manufactured by Xanar, Inc., Colorado Springs, Colorado. The current system is in reality a hollow fiber guide measuring 77 cm in length with a total outer diameter measuring 2.0 mm and an inner cross section of 0.5 mm per side (Fig. 1). The fiber is constructed of beryllium copper with a cladding of kymar. Interchangeable (disposable) tips measuring 1 mm outer diameter by 20 mm long are inserted into the operative end of the fiber and serve to protect the interior from particulate matter deposits (Fig. 2). All fibers can be sterilized in plastic tubes by standard gas sterilization techniques that use ethylene oxide. The fiber is flexible to the extent that it can subtend an arc of 90 degrees without loss of function. The fiber is attached to the



Fig. 1. Flexible carbon dioxide laser fiber measuring 2 mm outer diameter with a 1 mm outer diameter disposable tip.

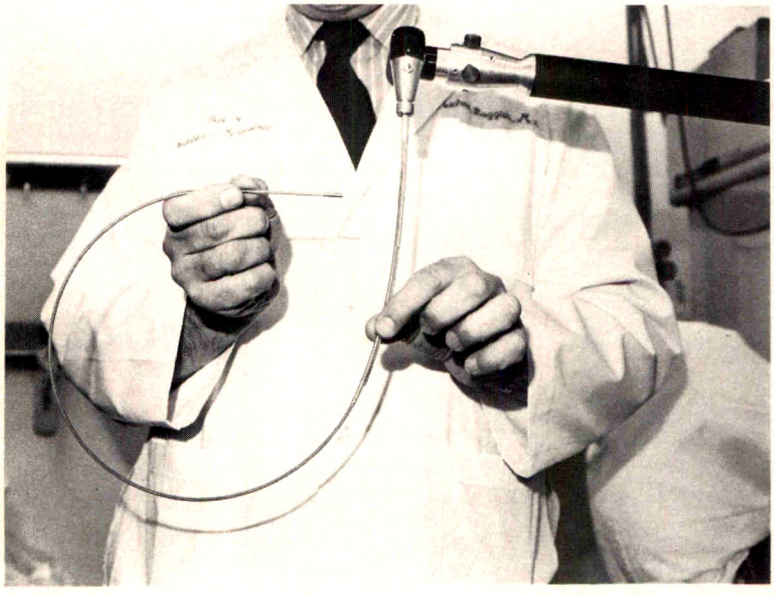


Fig. 2. The fiber is very flexible and can subtend an arc of 90 degrees. Here it is shown attached to the laser head by an intervening special arm fitted with an articulating knuckle.

laser head of a Xanar XA-20 carbon dioxide laser (maximum output 20 W) via the interposition of a special arm measuring 58 cm. The terminus of the arm is fitted with a mobile knuckle and a gas inlet. The arm also incorporates a focusing lens and a simple alignment mechanism. The latter can easily be adjusted before initiation of the operative procedure without having to actually attach the fiber to the arm. As a result of previous laboratory investigations, blower carbon dioxide flow is adjusted to deliver gas through the fiber at a flow rate of 1900 cc/min (Fig. 3).

A small trocar (Carl Storz, Culver City, Calif.) measuring 14 cm long by 3.5 mm outer diameter is used to facilitate entry of the fiber into the abdominal cavity.

Operative procedure

Table I lists the patients treated, together with their respective diagnoses. A total of 12 women underwent carbon dioxide laser laparoscopy with the flexible fiber from March 1986 to February 1987. Each patient had previously read and signed a detailed informed consent. All operations were performed under general endotracheal anesthesia. Before surgery the vagina and abdomen were prepared with povidone-iodine (Betadine). The urinary bladder was catheterized and emptied of urine. A single-toothed tenaculum was attached to the cervix, and a Cohen-Eder cannula (Eder Instrument Co., Chicago, Ill.) was placed in the cervix for manipulation. The abdomen was draped and the operative field was blocked with moist cotton towels. The fiber was attached to the laser arm, and the alignment was quickly rechecked by firing several spots into a wooden tongue depresser. A standard laparoscopy was performed with a touhy (epidural) needle to create a carbon dioxide pneumoperitoneum. Three liters of carbon dioxide gas were pumped into the belly by an insufflator delivering 2.5 L/minflow. A 7 mm outer diameter trocar was inserted through a 1 cm infraum-

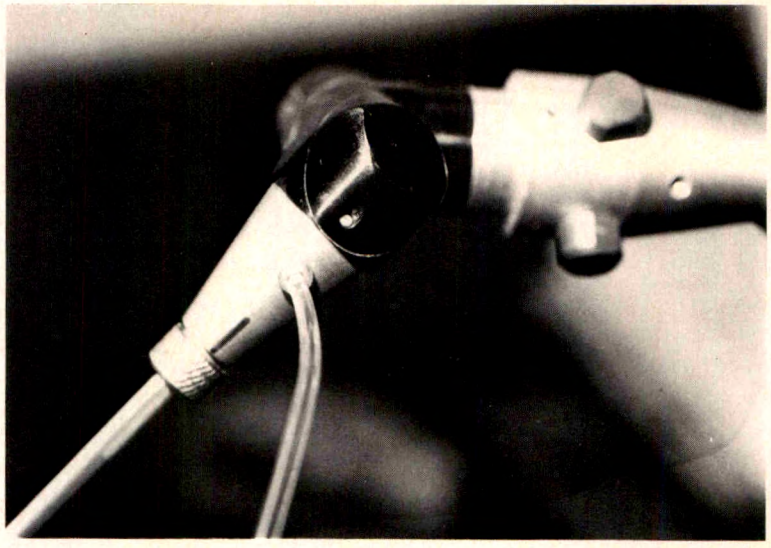


Fig. 3. A carbon dioxide blower tube is attached to an inlet on the knuckle pictured in Fig. 2. Carbon dioxide flow is adjusted to a rate of approximately 1900 cc/min. The constant flow of carbon dioxide gas keeps debris and vapor from obstructing the fiber lumen.

Table I. Twelve women treated by carbon dioxide hollow-fiber and laparoscopy, 1986 to 1987

Patient No.	*Diagnosis**	*Power (W)*	*Procedure*
1	Endometriosis*	10	Vaporization of implants
2	Endometriosis*	10-15	Vaporization of implants
3	Endometriosis*	10-20	Vaporization of implants, drainage of endometrioma
4	Endometriosis*	10-15	Vaporization of implants, lysis of adhesions
5	Pelvic pain	20	Lysis of adhesions
6	Infertility/hydrosalpinx	20	Neosalpingostomy, lysis of adhesions
7	Endometriosis/myomata†	10-20	Vaporization of implants, vaporization of myoma (2 cm)
8	Endometriosis†	20	Drainage of endometrioma, vaporization of implants
9	Myomata/infertility	20	Vaporization of myomata
10	Endometriosis*	10-20	Vaporization of implants, lysis of adhesions
11	Endometriosis*	10-20	Vaporization of implants
12	Endometriosis*	10-20	Vaporization of implants

*Stage II.
†Stage III (American Fertility Society Classification).

bilical incision followed by a 6.5 mm outer diameter laparoscope (Carl Storz). A second puncture was placed just below the pubic hair line to the left of midline by means of a 7 mm trocar. A combination smoke evacuator irrigation cannula (Eder Instrument Co.) was inserted through this entry port. Inspection of the pelvic viscera was then carried out. Next, a 3.5 mm trocar was inserted approximately 3 to 4 cm below the umbilicus to the right of the midline through which the laser fiber was passed. The carbon dioxide flow through the fiber was activated and was sufficient to maintain abdominal distension throughout the procedure. Alternatively, a standard 9 mm single puncture laparoscope with a 3.5 mm operating channel (Richard Wolf) was used in four cases. The laser fiber was conveniently inserted through the operating channel. When the operator fired the laser, smoke was also discharged from the field. The power was turned up to 10 to 20 W (Table I).

The fiber was brought to within 2 to 3 mm of the lesion to be treated, and the laser was then fired, usually at a continuous time setting. For strategically located lesions, the laser was time gated at 0.2 second for more accurate control. The second puncture irrigating-evacuation cannula could also double as a backstop for the laser beam. The actual fiber was stiff enough to perform fine manipulations of structures with the ab-

domen. The highest power settings were used to cut adhesions and vaporize myomas.

Endometriomas were opened at 20 W and the contents were drained. The fiber could be inserted directly into the cyst and the lining was vaporized by reducing the power to 10 W. Endometrial implants were vaporized at 10 W at time-gated 0.1 to 0.2 bursts or at continuous time exposure. Seedling myomas could be vaporized outright at 20 W or the serosa incised at 10 to 15 W followed by capsular vaporization at 15 W. The myoma was then teased out with the fiber and grasping forceps. Adhesions were eliminated with the evacuator-irrigation probe as a backstop and cutting the adhesions at 20 W. As in the rabbit studies, maximal impact power was observed at a distance of 2 to 3 mm.

When the procedure was finished the char material was washed away from the ablated areas with heparinized lactated Ringer's solution (5000 U of heparin/L of sterile lactated Ringer's solution). Excess irrigation fluid was sucked out of the cul de sac. The pneumoperitoneum was allowed to escape from the abdominal cavity and all instruments were withdrawn. The wounds were closed with interrupted 3-0 vicryl sutures.

When lesions located on the right portion of the pelvis could not be conveniently treated by the initial introductory site of the fiber, a third or even fourth stab could be placed in the midline or to the left of the midline to allow better positioning. The fiber could be manipulated within the abdomen to better impact a lesion by using a grasping forceps to induce a small bend. This unique maneuver is a tremendous advantage for performing very precise laparoscopic surgery. Virtually any lesion that can be seen can also be vaporized or incised. When bleeding occurs, the fiber can be withdrawn to a distance of 4 to 5 mm and coagulation brought about. In addition, the carbon dioxide flow through the fiber helped blow away blood while the bleeding point was coagulated.

Results

Ablative lesions produced by the carbon dioxide laser fiber were identical to those produced by the laser free-hand piece. At a target distance of 2 mm, a spot measuring 0.8 mm was created. The histologic findings of these lesions were previously described.[1] A reduction of laser power was observed when gas flow through the fiber was increased. Flow rates above 2000 cc/mm produced an estimated 40% reduction of power. At flow rates of 2000 cc, power was estimated to be reduced by 25%. When no gas was instilled through the fiber, laser smoke and moisture were deposited within the lumen of the guide. This led to markedly reduced laser power and eventually to a functionless fiber. Interior heat resulting from the laser impacting on the debris also caused damage to the interior-coating material. Certain fiber configurations also led to a reduction of laser power. The worst configuration was reverse S shaped and reduced power by approximatley 50%. Laboratory evaluation that used a power meter confirmed the extent of power reduction. The fiber regained power on straightening, that is, the reduction was in no way permanent. One fiber could be used repeatedly without loss of power or mode. During the study, fibers were alternated, that is, one was gas sterilized while the other was in use except on occasions when more than one laser case was posted during the same day. The removable fiber tips were replaced liberally; sometimes two were used during a single case.

During the laparoscopic procedure, the carbon dioxide flow through the fiber was more than adequate to maintain the pneumoperitoneum. In fact no gas was ever delivered during laser surgery through the laparoscopic insufflator. Because of this constant flow of gas, the assistant maintained a vigil for overdistension of the abdominal wall and allowed gas to escape on a timely basis.

Of 12 cases treated, two pregnancies have been reported. One patient treated early in the series was delivered. One woman is currently pregnant. Four patients were treated for pelvic pain and have reported an improvement in their symptoms. One of these women underwent drainage of an endometrioma and was recently seen. Although this woman reported general relief of pain, she nevertheless complained of deep pressure within the pelvis and is currently being evaluated for a recurrence of her endometrial cyst. The two women who had vaporization and/or excision of myomata had surgery too recently to obtain suitable follow-up information.

All patients were examined 4 to 6 weeks after surgery. All wounds healed nicely. The patients were uniformly pleased because of the good cosmetic results from the small scars that resulted from the 3 mm stab wounds made for the entry of the small trocar through which the fiber gained access into the abdominal cavity. No complications related to the use of the carbon dioxide laser fiber were observed or reported during this study.

Comment

This study demonstrated several advantages of a carbon dioxide laser fiber system when compared with conventional, rigid, metal tube delivery. The principal benefits were the relatively small-fiber diameter and flexibility of the fiber. The ability to use the fiber in conjunction with single-puncture laparoscopy while enjoying the advantages of visualizing the fiber and the target at all times added significant safety factors to this methodology.

The construction of the fibers used in this study, that is, beryllium copper, offered greater flexibility and du-

rability compared with the dielectrically coated aluminum fiber used in our previous study. In that earlier study, once fiber alignment was completed, further realignment was unnecessary. No helium neon beam was present and it was not needed. The major disadvantage of this system centered on the overall short length of the fibers. Longer fibers would be advantageous, since the laser arm could be placed in a more remote location from the operative field. The crater diameter, depth, and peripheral coagulation effect were at all times predicated on the fiber-to-target distance and the laser power setting. The stiffer fiber construction had certain advantages when compared with smaller diameter, highly flexible quartz fibers.[5,6] The ability to manipulate structures between lasering cycles provided better exposure and proved to be a highly advantageous operative maneuver.

The safety aspects of carbon dioxide laser fiber technology are not inconsiderable and provide laser laparoscopy with an extra margin of security for both the patient and the surgeon. Based on the data obtained in this study and previous work, the carbon dioxide laser fiber should offer a favorable alternative for laser and operative endoscopy. The technique is easier to learn than those currently available for laser laparoscopy.

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Decreased elastic fibers and desmosine content in incompetent cervix

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Incompetence of the uterine cervix is a syndrome of painless, progressive dilatation and effacement occurring between the sixteenth and twenty-fourth weeks of gestation that represents abnormal functioning. It may serve as a model to elucidate normal function. Because the incompetent cervix results in painless opening of this organ without uterine contraction before term gestation, it is considered one of the causes of midtrimester spontaneous abortion, habitual spontaneous abortion, and early preterm labor. Untreated, it leads to rapid expulsion and often death of the fetus. We used light microscopy to compare decreased elastic fibers in incompetent cervices with those of normal nonpregnant and pregnant cervices. Morphologic analysis of this difference was extended to biochemical quantification of elastin content in one patient with cervical incompetence. The decrease in elastin suggests that one function of cervical elastin may be to maintain a closed and undilated cervix throughout gestation. There may be a relationship between changes in cross-linked elastin and the incompetent cervix; further studies are therefore indicated. (Am J Obstet Gynecol 1987;157:1134-9.)

Key words: Incompetent cervix, elastin, elastase, gestation, parturition

Incompetence of the uterine cervix is a syndrome defined as painless, progressive dilatation and effacement that occurs between the sixteenth and twenty-fourth week of gestation.[1, 2] It is considered one of the causes of habitual spontaneous abortion, midtrimester abortion, and early preterm labor. Untreated, it leads to sudden rupture of the amnion and chorion, followed by rapid expulsion of the fetus.[3, 4] Because this syndrome represents an abnormal function of the cervix, it may serve as a model to elucidate normal function.

We have previously reported that elastin is present in the uterine cervix and can be assayed by measurement of the elastin-specific cross-links desmosine and isodesmosine.[5, 6] We have also established that the elastic fibers are organized in a band 20 to 30 μm thick, parallel to and between the collagen fibers from the periphery to the external os and toward the internal os.[7] These fibers are organized in such a way that after effacement or thinning of the cervix before active labor, they would be just beneath the fetal presenting part. It is of considerable interest to determine the amount of elastin in the clinically incompetent cervix, as any deviation from normal could suggest a role for elastin in gestation and parturition.

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Methods

Diagnosis of incompetent cervix. For the purposes of our study, the criteria for inclusion in the diagnostic category of incompetent cervix were documented evidence of progressive, painless dilatation without uterine contraction, and effacement of the cervix in the late first or second trimester, a history of previous painless dilatation of the cervix and/or a history of cervical trauma, such as a difficult dilatation and curettage, and a cerclage in place before the time of delivery.

Human tissue sources. All subjects were recruited into the study after individual informed consent was given and the approval of the institutional review board for research involving humans was granted. In the study period described, two patients with incompetent cervix declined to participate.

Six patients who met the criteria for incompetent cervix underwent anterior, full-thickness 2 by 2.5 cm wedge biopsy from the external os toward the anatomic internal os immediately after delivery. These were samples 1 to 6.

Six uterine cervical tissue specimens were obtained from women either at the time of hysterectomy or as a full-thickness 4 by 5 cm anterior wedge biopsy from the external os to the internal os immediately after

delivery. These were samples 7 to 12. Sample 7 consisted of the whole cervix of a woman at 40 weeks of normal gestation who underwent a cesarean hysterectomy. The cervix was identified and dissected free from the lower uterine segment. Sample 8 consisted of anterior and posterior wedges from the hysterectomy specimen of a woman who had a ruptured cornual pregnancy at 16 weeks' gestation. Sample 9 consisted of anterior and posterior wedges from the cervix of a woman 30 weeks pregnant who had a cesarean hysterectomy. Tissue sample 10 was the whole cervix of a nonpregnant woman who had a hysterectomy for benign disease. Sample 11 was obtained from a woman who had a cerclage at 12 weeks' gestation and was delivered at 38 weeks; before placement of the cerclage she had had uterine contractions noted by both palpation and electronic fetal monitoring and thus did not have classical incompetent cervix. Sample 12 was an anterior wedge of cervix obtained immediately after delivery from a woman with a classic history of incompetent uterine cervix, including painless, cervical dilatation in four previous pregnancies. She had a cervical cerclage at 12 weeks.

Seven additional samples (13 to 19) were all obtained from nonpregnant patients at the time of hysterectomy for benign disease.

Light microscopic studies. Cervical biopsy tissue specimens 1 to 6 obtained from patients who met criteria for incompetent cervix and from samples 7, 8, 9, and 13 to 19 were immediately fixed in phosphate-buffered formalin. These tissues (6 mm thick) were processed routinely for light microscopy. They were stained by the modified iron-hematoxylin stain for elastin described by Musto,[8] which reveals sharply outlined elastin fibers. Briefly, tissues were deparaffined and hydrated. They were mordanted in a saturated solution of alcoholic Bouin's fluid at 55° to 60° C for 10 minutes. The sections were then washed in tap water, rinsed briefly in distilled water, and stained with the elastin stain for 45 minutes. After staining, the sections were washed in tap water, rinsed in distilled water, and stained with woodstain scarlet for 5 minutes. After a second water wash the sections were treated with 2% phosphotungstic acid (2 minutes) and with 5% acetic acid (5 minutes), rinsed in distilled water, dehydrated in alcohol, then stained in 1% alcoholic saffron (5 minutes). Sections were then dehydrated and cleared before mounting. With this stain the elastic fibers stain black and are sharply outlined, nuclei stain dark purple to black, muscle cells stain pale red, and collagen stains yellow. Multiple serial sections were prepared at various tissue levels of the cervix. All sections were thoroughly searched under the high-power occular setting for elastic fibers. Results were compared as number of fibers seen in at least 10 high-power fields.

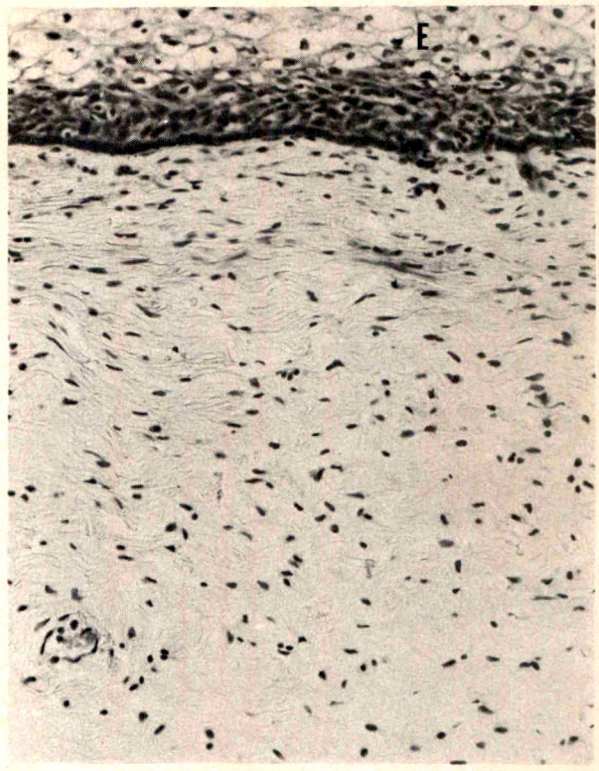


Fig. 1. Photomicrograph of an incompetent cervix. No elastic fibers are noted. None of the samples of incompetent cervix (with one exception) showed any elastic fibers. *E*, Epithelium. (Original magnification ×310.)

Biochemical analysis

Extraction of crude connective tissue. Tissues from the six cervical specimens 7 to 12 derived from five normal cervices and one incompetent cervix were frozen immediately at −20° C and then stored until processing. After thawing, the tissue was trimmed, weighed, and minced thoroughly with scissors. The tissue was then homogenized in a blender with 1 mol/L sodium chloride, washed and resuspended in 1 mol/L sodium chloride, and stirred overnight at 5° C. This step was repeated a total of five times. After the fifth centrifugation, the residue was washed three times in deionized water and lyophilized. The dry powder was then extracted three times in cold *n*-butanol and three times in cold acetone to remove lipids and was then dried overnight. This material was designated crude connective tissue.

Preparation of samples for biochemical assays. Ten milligrams of each sample (with the exception of sample 6, in which 2.5 mg was used) was hydrolyzed in 1 ml of 6N hydrochloric acid for 48 hours at 116° C. The hydrolysate was filtered, diluted with 20 ml of water, and lyophilized. The residue was dissolved in 10 ml of water and lyophilized to remove excess hydrochloric acid, after which the process was repeated. The hy-



Fig. 2. Photomicrograph of sample 3 from a subject who met the criteria for incompetent cervix. Note that the elastic fibers are decreased in number compared with those of a normal cervix and they appear to be broken *(arrow)*. *E*, Epithelium. (Original magnification ×310.)

drochloric acid–free residue was dissolved in 3 ml of water.

Radioimmunoassay for desmosine. A 0.1 mg aliquot of the hydrolysate was used for the desmosine assay. This aliquot was diluted with isotonic veronal-buffered saline solution at pH 7.2 and was assayed for demosine by a monospecific radioimmunoassay as previously described.[9] Briefly, aliquots of hydrolysate were mixed with rabbit anti–desmosine serum and incubated at room temperature for 15 minutes. Radioactive desmosine labeled with Bolton-Hunter-^{125}I reagent (New England Nuclear, Boston, Massachusetts) was added and further incubated for 1 hour at 30° C. The antibody and desmosine complex was separated by centrifugation in the presence of polyethylene glycol according to the method of Desbuquois and Aurbach.[10] One hundred microliters of bovine γ-globulin (1.75 mg/ml in 0.85% sodium chloride) was added as a carrier, followed by 0.5 ml of cold 25% polyethylene glycol solution (in 0.1 mol/L sodium borate, pH 8.0). The precipitate was separated by centrifugation at 4000 rpm at 4° C for 30 minutes and then washed once with 0.5 ml of cold 12.5% polyethylene glycol solution. Radioactivity of the precipitate was measured in a gamma counter. Standard desmosine solutions were used from 0.5 to 16 pmol in 100 μl of isotonic veronal-buffered saline solution. Each assay was repeated on each sample from five to 16 times.

Isolation of elastin. Approximately 200 mg of samples 7 and 9 (crude connective tissue) was extracted with 5 ml of 0.1 mol/L sodium hydroxide at 98° C for 45 minutes according to the method of Lansing et al.[11] The residue was then hydrolyzed with 6N hydrochloric acid and analyzed for desmosine by the radioimmunoassay.

Calculation of elastin percent in tissue. The amount of elastin was calculated by converting desmosine at a value of 13.9 nmol desmosine per milligram elastin. Results are expressed as desmosine or elastin per milligram of dry, defatted insoluble residue. Percent elastin is expressed as percent per crude connective tissue on the basis of desmosine content.

Results

In five of the six anterior wedge biopsy samples derived from the cervices of women with the clinical criteria of incompetent cervix, light microscopy revealed no elastic fibers (Table I, Figs. 1 and 2). The gestational ages of the pregnancies ranged from 8 to 40 weeks. A sample obtained at 25 weeks' gestation revealed elastic fibers; however, these fibers were decreased in number and were broken.

All 10 of the samples from normal cervices clearly showed elastic fibers that appeared to be oriented in a bandlike configuration, as recently demonstrated in this laboratory.[7] The presence of elastic fibers was not affected by the age of the woman or her parity.

Fig. 1 is a photomicrograph of an incompetent cervix showing the absence of elastic fibers, and Fig. 3 is a normal cervix photomicrograph with clear evidence of elastic fibers. Fig. 2 is a photomicrograph of the cervical tissue of a patient who had clinical evidence of incompetent cervix. It reveals broken elastic fibers that appear decreased in amount.

Results of the biochemical studies demonstrate that the desmosine content of the cervical tissue ranges from 102 ± 4 (SEM) to 287 ± 12 (SEM) pmol/mg, the lowest amount being present in the tissue from a woman with incompetent cervix as compared with normal cervical tissue. This represents a considerable decrease in mature cross-linked elastin in the incompetent cervix (Table II, Fig. 4). The nonpregnant patient had the highest amount of elastin, as calculated by both percent elastin in the crude connective tissue fraction and by picomoles desmosine per milligram tissue, almost double the amount found in cervices from pregnant patients. Each sample was assayed repeatedly, as noted in Table II.

The results obtained on samples 7 and 9 by extraction of the crude connective tissue by the Lansing et al.[11]



Fig. 3. Photomicrograph of a normal cervix. *Arrow* points to an elastic fiber. Note the numerous fibers throughout the cervical stroma. *E*, Epithelium. (Original magnification ×310.)

procedure followed by the desmosine radioimmunoassay were similar to those obtained with the direct hydrolysate of crude connective tissue, indicating that there was no difference in the amount of this specific cross-link in the crude connective tissue and in the pure elastin extracted. These results confirm that the radioimmunossay was specific for desmosine and did not cross react with any collagen cross-links.

Comment

We used the incompetent cervix as a model to determine the role of elastin in the cervix during gestation. The diagnosis of incompetent cervix is suspected by a history of repeated painless second-trimester pregnancy loss and is confirmed by the clinical finding of a patent cervical os in pregnancy.[12-15] It is quite rare and it is suggested that the true incidence lies between 1 in 1000 and 1 in 2000 live births.[16] Past histories of women with incompetent cervix include cervical operation, especially amputation; forcible dilatation of the cervix for diagnostic curettage or termination of pregnancy; trauma to the cervix during childbirth; and exposure in utero to diethylstilbestrol.[15] In approximately 2% of women who have been diagnosed as having an incompetent cervix, there is no apparent cause.[15] Theoretically, it could be postulated that trauma to the cervix destroyed the elastic fibers, which run in a band just under the epithelium of the endocervical canal.[7] This would explain why the incompetent cervix traditionally included a history of cervical trauma.[15] Our tissue sample 12 was obtained from a woman with a history of

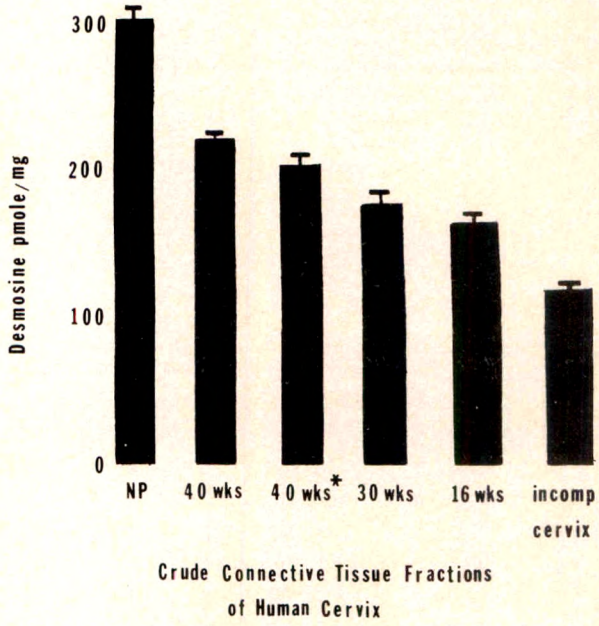


Fig. 4. Amount of cross-linked elastin in human uterine cervix expressed as picomoles desmosine per milligram crude connective tissue. *Sample from a patient with pregnancy loss without incompetent cervix.

three midtrimester pregnancy losses after a spontaneous abortion at 12 weeks. All of these losses followed painless dilatation of the cervix. Because all wedge biopsy specimens were obtained from the anterior and in some cases from both the anterior and posterior cervix in the area of the endocervical canal, where the

Table I. Elastic fibers in human cervix

Sample	*Gravidity and parity*	*Gestational age (wk)*	*Elastic fibers*
Incompetent cervix tissue			
1	G7P1	8	Absent
2	G4P0	22	Absent
3	G3P0	25	Decreased and broken
4	G8P4	32	Absent
5	G3P0	40	Absent
6	G2P0	40	Absent
Clinically normal cervix, pregnant patients			
7	G3P2	40	Present
8	G2P1	16	Present
9	G3P2	30	Present
Clinically normal cervix, nonpregnant patients			
13	G3P3		Present
14	G3P3		Present
15	G0P0		Present
16	G6P4		Present
17	G0P0		Present
18	G0P0		Present
19	G0P0		Present

Table II. Desmosine and elastin contents of cervical crude connective tissue

Tissue sample	*Gestational age (wk)*	*Type of cervix*	*Desmosine (pmol/mg tissue)*		*Elastin* (%)*
			Mean ± SEM	*No. of assays*	
7	40	Normal	206 ± 3	9	1.50
8	16	Normal	154 ± 7	6	1.10
9	30	Normal	168 ± 8	7	1.20
10	Nonpregnant	Multiparous patient	287 ± 12	16	2.06
11	40	Cervix from patient with history of pregnancy loss	192 ± 7	8	1.38
12	40	Incompetent cervix	102 ± 4	5	0.73

*Calculated by converting desmosine to elastin on a basis of a value of 13.9 nmol desmosine per milligram elastin.

elastic fibers are normally located, the decrease in elastin content cannot be considered to be due to sampling errors. Whether the decreased elastin content is due to loss of elastin because of trauma or to an unknown cause in these cases cannot be determined at this time. Nevertheless, it is obvious that the clinical course in this study of the pregnancies complicated by incompetent cervix was very different from that of normal gestation in which cervical elastin was not decreased.

Elastin has been shown thermodynamically to effectively store elastic energy and release it as well as to stretch and retract, and is considered to have high rebound resilience or reversible extensibility.[17, 18] This property would be important in maintaining a closed cervix during gestation.

Unfortunately, it was not possible to obtain tissue from a second woman with incompetent cervix ample enough for biochemical analysis in part because of the rarity of the snydrome and the reluctance of pregnant subjects to undergo cervical biopsies.

A fairly rare clinical syndrome, the incompetent cervix was used as a "natural" experimental model to help elucidate the role of elastin in the retention of the fetus in utero. The subjects were compared with women at various weeks of gestation who had normal cervical functioning. Correlation of abnormal function of the cervix with the histologic findings of absent or decreased elastic fibers as opposed to a bandlike configuration of these fibers[7] and biochemical demonstration of decreased desmosine content in the incompetent cervix suggests that the decrease in elastin is real. It further suggests that the decrease in desmosine is not caused merely by decreased rates of cross-link formation. These differences were noted throughout all gestational ages. It is important to note that all cervical tissue studied by the radioimmunoassay was from multiparous women. Labor status did not seem to affect differences within the cervical tissue of the pregnant subjects, since specimens 7, 11, and 12 were obtained from women after the onset of physiologic labor, while spec-

imens 8 and 9 were obtained before labor. Sample 10, which had the highest amount of elastin, was obtained from a nonpregnant multiparous woman. Because leukocyte elastase is present in the cervix during gestation, presumably elastin is degraded in cervical tissue during pregnancy; this could account for the difference. Samples 7, 8, and 9 were obtained from cesarean hysterectomy specimens and the whole cervix was thus available for analysis. These samples could be studied by both biochemical and histologic methods. The other samples, which were from wedge biopsies, provided only sufficient tissue for either biochemical or histologic studies, not both.

Because women with the clinical entity of incompetent cervix have a clear, recognizable abnormality in the physiologic function of cervical tissue in pregnancy, the fact that the incompetent cervices we studied have absent or decreased elastic fibers compared with tissue from women with normal gestation indicates that elastin may contribute to the retention of the fetus throughout gestation until the onset of labor. This conclusion is further supported by the biochemical quantitation of decreased desmosine in a patient with cervical incompetence.

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Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes

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To improve the accuracy of fetal weight estimation by ultrasound measurement, especially in low birth weight infants, including those with intrauterine growth retardation, two new formulas were developed from a new point of view. The formulas were established by (1) actual measurements of the volumes of the neonatal head and trunk with limbs, (2) analysis of the relationship between the sizes of the parts of the fetal body measured by ultrasound examination before birth and the volumes of the parts of the neonatal body, and (3) calculation of the specific gravities of the parts of the neonatal body. The prospective study showed a high correlation between estimated weights and actual birth weights with a small range of error in infants weighing between 450 and 4800 gm. (AM J OBSTET GYNECOL 1987;157:1140-5.)

Key words: Fetal weight estimation, ultrasound, specific gravity, intrauterine growth retardation

Although many formulas for fetal weight estimation have been reported,[1-5] their accuracies are not sufficient in cases of low birth weight infants, especially those with intrauterine growth retardation (IUGR).[1-5] The reasons for incorrect estimations are as follows: (1) Most formulas were made by the regression method that uses birth weights and values for each fetal part such as biparietal diameter (BPD) and trunk diameter, obtained through ultrasonic measurements before birth. Accordingly, these formulas use the heavier coefficients for BPD, which can be measured with a smaller range of error than can trunk diameter. (2) The accuracy of any formula made by the regression method depends on the number of cases taken into consideration in devising formulas. Such formulas are not suitable for fetuses with IUGR, whose proportions are different from those of normal fetuses, because the samples used include few cases of IUGR.

For these reasons, we tried to establish more logical formulas that considered the specific gravities and the volumes of neonates.

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Method

Ultrasonic measurements. Ultrasonic measurements of the fetuses included BPD, APTD (anteropostero trunk diameter), TTD (transverse trunk diameter), SL (spine length), and FL (femur length).

Each measurement was performed according to the following profile. BPD was measured from the outer edge of the anterior skull to the inner edge of the posterior skull on a section in which the cavity of septum pellucidum, the cisterna corpora quadrigemina, and the midline echo were clearly visualized.[6] APTD and TTD measurements were performed at right angles just vertical to the descending aorta where the umbilical vein was clearly visualized at a point one third to one fourth its length from the anterior abdominal wall.[6, 7]

FL was determined by measuring the length of the ossified part of the femur bone.[6]

SL was determined by measuring the spine from L-3 to T-6. L-3 was identified by visualization of the promontorium.[8]

Principle of the new formula for fetal weight estimation. The principle on which the new formula is based is illustrated in Fig. 1. The fetal body is divided into two components, the head and the trunk with limbs. The weight of the head is calculated by multiplication of the specific gravity and the volume of the head. The weight of the trunk with limbs is calculated in the same way. The volumes of each component are calculated by multiplication of the coefficients (A and B) and the parameters (BPD^3 APTD × TTD × SL)

$$\text{Fetal weight} = \underbrace{\text{Specific gravity of the head} \times A \times \underbrace{BPD^3}_{\text{Volume of the head}}}_{\text{Weight of the head}} + \underbrace{\text{Specific gravity of the trunk} \times B \times \underbrace{APTD \times TTD \times SL}_{\text{Volume of the trunk}}}_{\text{Weight of the trunk}}$$

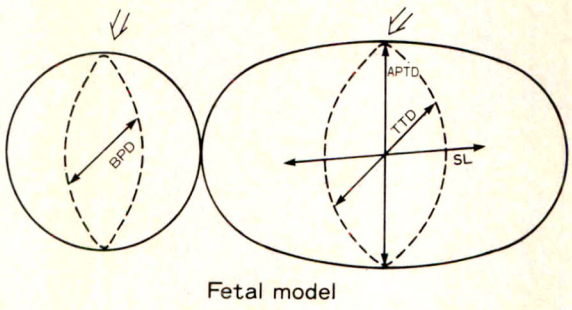


Fig. 1. Principle of the formula for fetal weight estimation. BPD^3 and APTD × TTD × SL were used as three-dimensional parameters of the head and the trunk, respectively. Coefficients A and B are the ratios of the volumes and the parameters of each part. The trunk includes the four limbs.

Table I. Number of cases entered in the prospective study and birth weight

Birth weight (gm)	*AFD*	*LFD*	*HFD*	*Total*
0-999	5	12	0	17
1000-1499	13	21	0	34
1500-1999	29	47	0	76
2000-2499	54	87	0	141
2500-2999	141	13	0	154
3000-3499	143	0	0	143
3500-	65	0	27	92
Total	450	180	27	657

AFD = Appropriate for date; LFD = light for date; HFD = heavy for date.

(Fig. 1). The coefficients are equal to the ratios of volumes and parameters. Accordingly, the weight of each component is calculated by multiplication of the specific gravity, the coefficient, and parameter.

Determination of specific gravities, coefficients (ratio of volume/parameter), and establishment of formula. The specific gravities of each part and the relationship between the volumes and the parameters were determined by the following procedures.

A. Each part of the fetuses was measured by ultrasound examination within 2 days before birth.

B. Birth weights were measured immediately after delivery.

C. The actual volumes of the heads and the trunks with limbs of the neonates were measured within 2 days after delivery. Thirty normal neonates with neither maternal complications nor any abnormalities of delivery were used. The mean gestational age was 39.1 ± 1.1 weeks (range 37 weeks, 2 days to 41 weeks, 2 days) and the mean birth weight was 3157 ± 306 gm (range 2524 to 3758 gm). The head was covered by special aluminum paper to make a mold of the head. The mold was then filled with small vesicles of forming polystyrol. The head volume was determined by measuring the volume of the vesicles with a graduated cylinder. The volume of the trunk with limbs was determined by measuring the volume of water that overflowed when the neonate was bathed. The accuracy of this method was confirmed by measuring several phantoms.

D. Using the values obtained from (A) and (B), the following ratios were calculated: A, volume of the head/BPD^3 and B, volume of the trunk with limbs/APTD × TTD × SL.

E. The specific gravities of each part were calculated by the multiple regression method using the results of (B) and (C).

F. A new formula was created that used each value obtained by these procedures. The volume of the trunk should normally be calculated with the three-dimensional parts of the trunk (APTD × TTD × SL, Fig. 1), but because it is sometimes difficult to measure SL, we also devised another formula in which FL is used instead of SL.

Prospective study. To confirm the accuracies of the

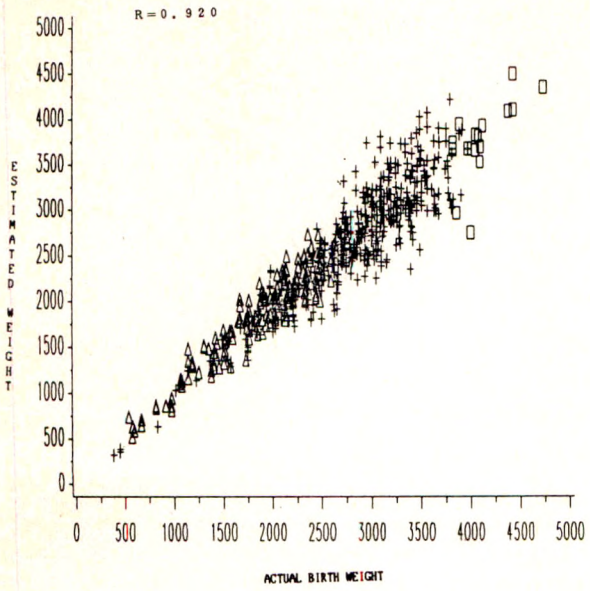


Fig. 2. Correlation between estimated weights and actual birth weights in formula 1: (△ :LFD, + :AFD, □ :HFD). (*LFD* = Light for date; *AFD* = appropriate for date; *HFD* = heavy for date.)

new formulas, a prospective study was done. The total number of cases studied was 657, including 180 cases of light for date. The gestational ages varied from 21 to 41 weeks, and the birth weights ranged from 450 to 4800 gm (Table I). All patients were ultrasonically examined within 7 days before delivery. With due regard to practical use of the formulas, the prospective study data were collected from examinations performed by several residents and specialists.

The correlations between estimated weights and actual birth weights and the errors related to birth weights were analyzed. The errors of estimation were expressed as percentages of actual birth weights: error of estimation (%) = (estimated weight − actual birth weight)/actual birth weight × 100.

All these statistical analysis were performed with a FACOM M-360 system at Tokyo University Hospital's computer center with SAS package software (version 5).

Results

Specific gravities and volume/parameter ratios. In the 30 cases studied, the specific gravities of the whole body, head, and trunk with limbs of the neonate were 1.132 ± 0.087 (mean ± SD), 1.085 and 1.132, respectively. The ratio of head volume to BPD^3, trunk with limbs volume to APTD × TTD × SL and trunk with limbs volume to APTD × TTD × FL were 0.990 ± 0.175 (mean ± SD), 2.573 ± 0.327, and 3.023 ± 0.401, respectively (Table II).

Table II. Specific gravity of neonate and relationship between volume and parameter of each part (mean ± SD)

SG of the whole body of neonate	= 1.130 ± 0.087
SG of the head	= 1.085*
SG of the trunk with limbs	= 1.132*
VH/BPD^3	= 0.990 ± 0.175
VT/APTD × TTD × SL	= 2.573 ± 0.327
VT/APTD × TTD × FL	= 3.023 ± 0.401
	(N = 30)

SG = Specific gravity; VH = volume of the head; VT = volume of the trunk with limbs.

*Specific gravity of the head and trunk with limbs were calculated values.

Table III. New formulas

(1) $1.07 \times BPD^3 + 2.91 \times APTD \times TTD \times SL$
(2) $1.07 \times BPD^3 + 3.42 \times APTD \times TTD \times FL$

New formulas. New formulas were based on the fetal model (Fig. 1). The values of specific gravities and coefficients (ratios of volumes/parameters) obtained from actual measurements were substituted. The first member of each equation means the weight of the head and the second that of the trunk with limbs (Table III).

Results of prospective study. The correlation between estimated weights and actual birth weights is shown in Fig. 2. The correlation coefficients of estimated weights and actual birth weights were 0.920 and 0.945 in formulas 1 and 2, respectively. The errors of estimation are shown in Fig. 3. The ranges of error for both formulas are shown in Tables IV and V. It was obvious that the errors were constant over a broad range of body weights from 450 to 4800 gm, and no differences of error were found among light for date, heavy for date, and appropriate for date. The error was <10% in about 70% of the cases. The error range was <15% in nearly 90% of the cases in all birth weight groups. The mean error ± SD was −0.4 ± 9.6% and −0.2 ± 9.5% in the light for date cases according to formulas 1 and 2, respectively.

Comment

Most previously reported formulas for estimating fetal weight were developed by the regression method.[1-5] We also used our regression method—based formula (estimated weight = $1.73 \times BPD^3 + 23.8 \times APTD \times TTD - 217$)[6-8] until we established a new one. Although the old formula was highly accurate in normal cases, it was inadequate for the weight estimation of low birth weight infants, especially those with IUGR.[9] Fig. 4 shows the errors observed in a prospective study performed that used our previous formula.

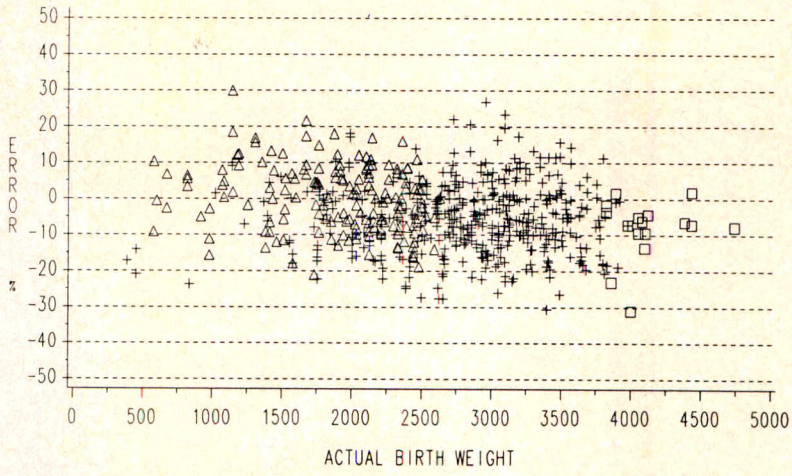


Fig. 3. Error of estimation in formula 1. (△ :LFD, + :AFD, □ :HFD). (*LFD* = Light for date; *AFD* = appropriate for date; *HFD* = heavy for date.)

Table IV. Range of error related to actual birth weight in formula I

Birth weight (gm)	*≦5%*	*≦10%*	*≦15%*	*≦20%*	*>20%*
0-999	33.3%	66.7%	75.0%	83.3%	6.3%
1000-1499	41.1%	70.6%	88.2%	100%	0%
1500-1999	40.4%	74.5%	89.4%	95.6%	4.3%
2000-2499	35.1%	74.0%	85.7%	96.1%	3.9%
2500-2999	49.3%	71.6%	86.6%	95.5%	4.5%
3000-3499	36.1%	65.3%	84.7%	94.5%	5.4%
3500-	48.5%	81.8%	91.6%	97.0%	3.0%
Light for date only	42.7%	79.6%	92.2%	98.1%	2.1%

Table V. Range of error related to actual birth weight in formula 2

Birth weight (gm)	*≦5%*	*≦10%*	*≦15%*	*≦20%*	*>20%*
0-999	33.3%	61.1%	77.8%	88.9%	11.1%
1000-1499	33.3%	72.7%	91.0%	97.0%	3.0%
1500-1999	45.5%	70.1%	91.0%	96.2%	3.8%
2000-2499	40.3%	72.7%	89.9%	96.4%	3.6%
2500-2999	47.1%	71.6%	87.1%	94.8%	5.2%
3000-3499	35.7%	68.2%	86.0%	94.3%	5.7%
3500-	47.4%	69.7%	81.6%	96.1%	3.9%
Light for date only	43.7%	76.3%	93.2%	97.9%	2.1%

It was obvious that patients weighing <2500 gm, including those with IUGR, produced heavier estimates than the actual birth weights, and the error of estimation was relatively large in these cases. Other formulas reported by Shepard et al.,[2] Walsof et al.,[1] and Hadlock et al.[3] showed the same tendency in our prospective study. The results of a prospective study that used the formula of Shepard et al. is shown in Fig. 5.

No improvement in accuracy was obtained as long as the regression method was used, because the tendency to produce heavier estimates in low birth weight infants was the result of the regression method. Because the regression model was based on patients with normal development, it is suitable for estimating normal cases but not for relatively rare abnormal cases such as IUGR.

We therefore established two new formulas that took into consideration fetal proportion, specific gravity, and volume. The results of a prospective study using these formulas showed that accuracy was significantly improved in low birth weight infants, especially those who were light for date (Tables IV, V and Fig. 3).

With our new formula, it was possible to estimate birth weights within a reasonably small range or error (15%) in nearly 90% of the cases. An error of ±10% to 15% is thought to be basically inevitable because the variance of values obtained through ultrasonic mea-

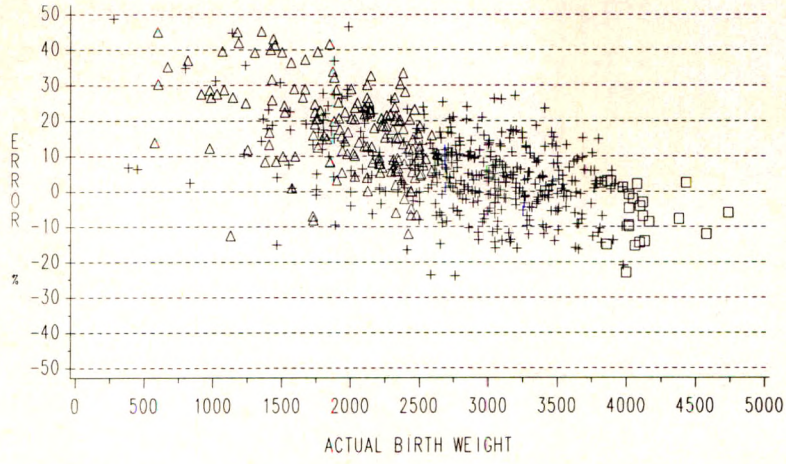


Fig. 4. Error of estimation in the formula made by the regression method: (△ :LFD, + :AFD, □ :HFD). (*LFD* = Light for date; *AFD* = appropriate for date; *HFD* = heavy for date.)

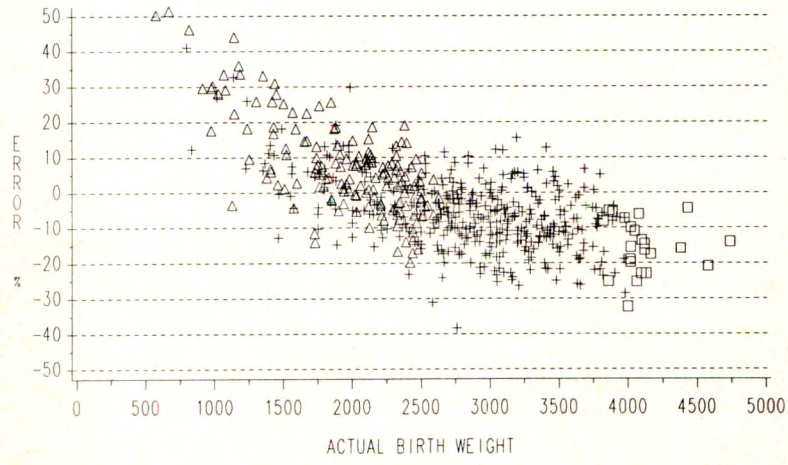


Fig. 5. Error of estimation when the formula of Shepard et al. was used. Heavier estimation was obvious in infants <2000 gm, especially those with IUGR. (△ :LFD, + :AFD, □ :HFD). (*LFD* = Light for date; *AFD* = appropriate for date; *HFD* = heavy for date.)

surements, especially those of soft tissue such as APTD and TTD, is of this magnitude.

Neonatal specific gravity is thought to change according to gestational age. Therefore, we tried to simulate the changes in specific gravity using the mean values of BPD, APTD, TTD, SL, and FL measured in 2313 cases and the mean birth weight for each gestational age on the assumption that the volume/parameter ratios were independent of gestational age. The simulation showed only slight changes in specific gravity according to gestational age. However, the improvement in accuracy achieved by correction of the formulas to adjust specific gravity according to gestational age was not significant. Furthermore, the corrected formulas were complicated (formulas 1 and 2 are multiplied by C: $C = 1.04 - 0.001 \times$ [34-gestational week]^2). The formulas with a constant specific gravity (Table III) are therefore thought to be better for practical clinical use.

In conclusion, it was confirmed that both new formulas based on fetal proportions, specific gravity, and the relationship between volumes and parameters are valuable for fetal weight estimation in low birth weight infants, especially those with IUGR, as well as normal fetuses.

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Soft tissue nuchal fold in the second-trimester fetus: Standards for normal measurements compared with those in Down syndrome

Beryl R. Benacerraf, M.D., and Fredric D. Frigoletto, Jr., M.D.

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There are several reports describing the sonographic sign of a thickened nuchal fold for the prenatal sonographic detection of Down syndrome in the second trimester. We prospectively obtained normative data on the nuchal folds of 303 consecutive normal fetuses undergoing genetic amniocentesis between 15 and 20 weeks' gestation. The data show that the width of the nuchal fold was consistently between 1 and 5 mm regardless of gestational age (15 to 20 weeks). (Am J Obstet Gynecol 1987;157:1146-9.)

Key words: Prenatal detection, Down syndrome, nuchal fold, ultrasound

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The sonographic finding of a soft tissue roll or skin thickening at the back of the fetal occiput is associated with the presence of Down syndrome in the second-trimester fetus.[1-3] In a series of 3825 consecutively scanned fetuses between 15 and 19 weeks' gestation, we found that this sign identified 42% (nine of 21 cases)

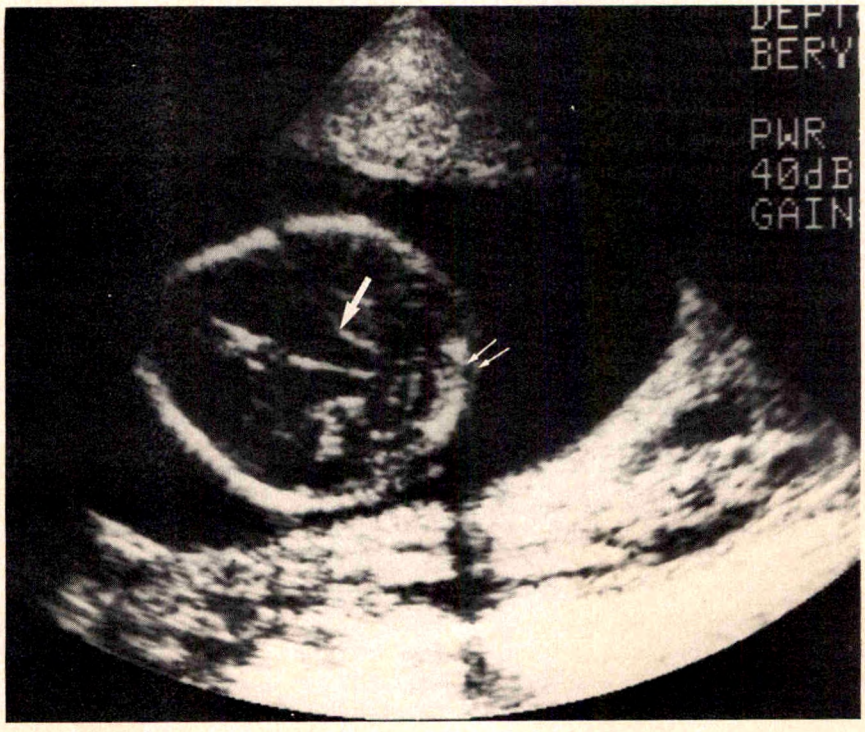


Fig. 1. Modified transverse view through the fetal head showing the thalami *(long arrow)* and the posterior fossa. The measurement of the nuchal fold is made from the outside of the occipital bone to the skin surface *(small arrows)*.

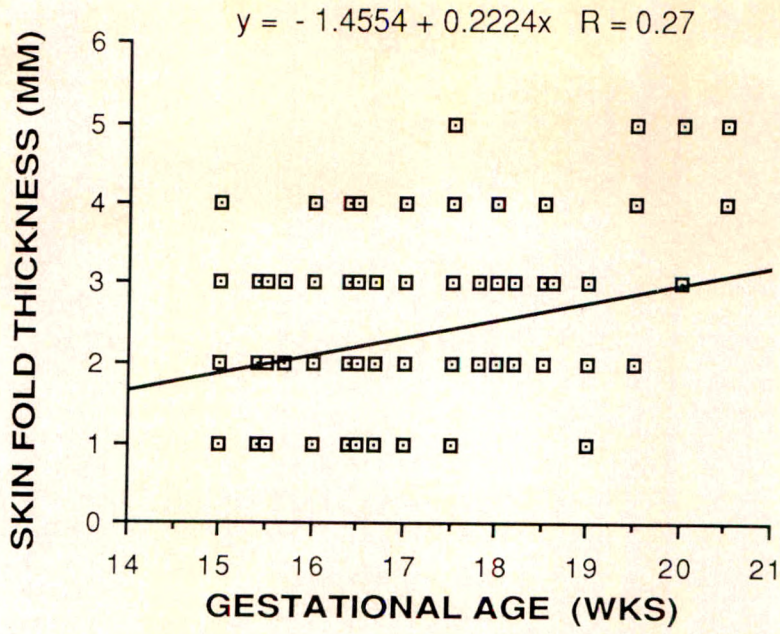


Fig. 2. Plot of gestational age versus nuchal skin fold thickness in millimeters. This demonstrates that there is no significant difference between the thickness of the skin fold in normal fetuses between 15 and 20 weeks. Note that each *square* represents several fetuses with the same measurement.

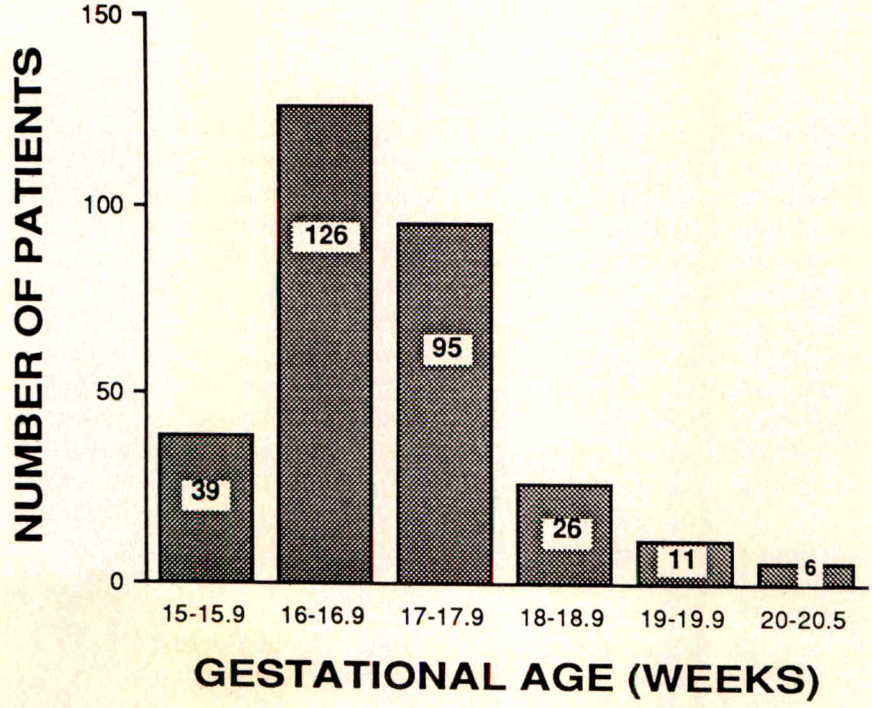


Fig. 3. Histogram showing the number of fetuses in each gestational age category within our sample of 303 consecutive fetuses at genetic amniocentesis.

of Down syndrome fetuses with a false positive rate of 0.1%.[1-3] In order to determine whether the thickness of the nuchal skin fold changed with increasing gestational age in fetuses between 15 and 20 weeks, 303 consecutive fetuses were studied at the time of amniocentesis. All were subsequently found to have normal karyotypes. The measurement of the nuchal fold was recorded prospectively in each case.

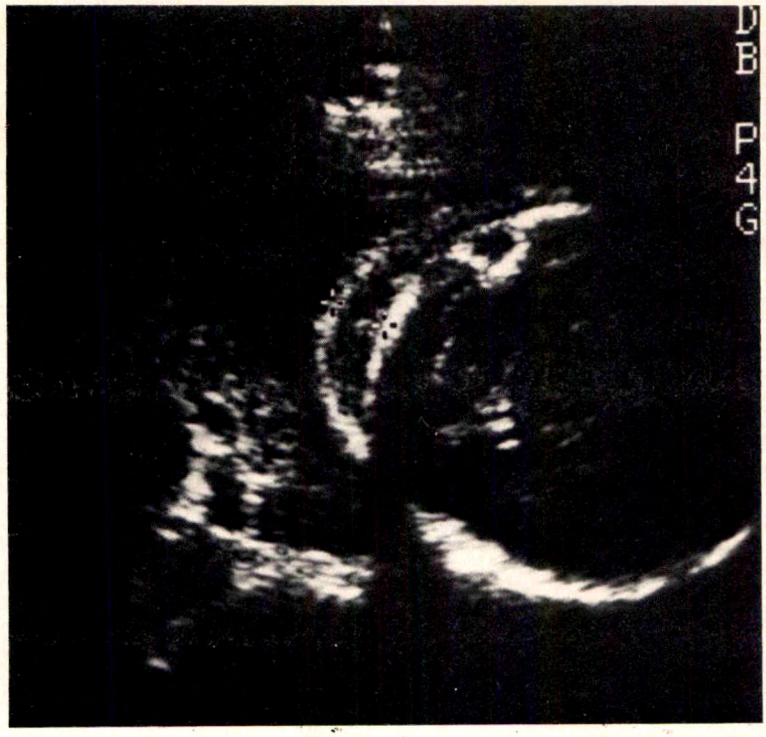


Fig. 4. Modified transverse view through the fetal head of a fetus with Down syndrome showing the measurement of the nuchal fold as made by the electronic calipers (7 mm). Please note that the posterior fossa and occipital bone are well seen in this view.

Material and methods

The soft tissues at the back of the fetal neck or occiput (nuchal fold) were measured prospectively in 303 consecutive fetuses presenting just before amniocentesis, between 15 and 20 weeks. Mothers were all at risk for Down syndrome either by being 35 years of age or more or by having a low maternal serum α-fetoprotein value. The scans were done with an Acuson 128 or ATL 300 real-time imaging system, with a 3.5 or a 3 mHz transducer, respectively. The view used to measure the back of the occiput was a modified transverse view through the fetal head, across the thalami, but angled slightly posteriorly to include the cerebellum and the occipital bone (Fig. 1). The soft tissue thickness posterior to the occipital bone was then measured, not including the bone, from the surface of the bone to the skin. Care must be taken not to measure the thickness from a plane taken below the level of the occipital bone, at the first cervical vertebra, or an incorrectly wide value will be obtained, leading to a false positive result. This assessment of the soft tissue nuchal fold was done as part of the routine measurements before the amniocentesis is performed.

Results

In these 303 fetuses measured between 15 and 20 weeks' gestation, the nuchal fold ranged between 1 and 5 mm, although most of the 5 mm measurements occurred after 19 weeks (Fig. 2). There was no significant difference in range or frequency of the measurements obtained between 15 and 20 weeks' gestation. The limited number of fetuses in the study between 19 and 20 weeks' gestation, however, do not represent conclusive data in this age group (Fig. 3). A number of attempts were made to fit these data to a curve, including linear, polynomial, and exponential algorithms, none of which yielded an r value >0.3. This strongly suggests that there are no significant differences between measurements of the nuchal fold behind the occiput between 15 and 20 weeks' gestation. None of the fetuses studied had a skin fold >5 mm.

Comment

Recent studies have shown that a nuchal fold of ≥ 6 mm in the second-trimester fetus has been associated with Down syndrome (Fig. 4). We have studied 3825 consecutive fetuses between 15 and 20 weeks' gestation undergoing genetic amniocentesis, which yielded 21 fetuses who had Down syndrome.[1-3] Nine of these 21 fetuses had a skin fold measuring ≥ 6 mm at the occiput. There were four cases where the measurement was ≥ 6 mm but the fetuses did not have Down syndrome (0.1% false positive). One of these four had an abnormal karyotype other than Down syndrome and the

others were normal. In order to further define this measurement of the nuchal skin fold in the fetus with Down syndrome, we have studied 303 consecutive normal fetuses between 15 and 20 weeks' gestation to establish the normal distribution of this measurement and to determine whether there are any differences with different gestational ages between 15 and 20 weeks' gestation. This study shows that the width of the nuchal fold was consistently between 1 and 5 mm regardless of gestational age. There were no false positive results in this study; however, because our rate of false positive results in our previous work has been 0.1%, one would extrapolate that only 0.3 fetuses would have false positive results in this group. It is therefore not surprising that no false positive nuchal fold measurements were discovered in this sample. It is of note that by far the largest number of fetuses in this study group of women undergoing genetic amniocentesis was between 15 and 19 weeks, as shown by the histogram (Fig. 3), and therefore further work would be necessary to confirm the findings on fetuses between 19 and 20 weeks' gestation because of the small numbers in that group.

It is crucial not to misuse the measurement that could lead to false positive results.[4] Too much angling in the ultrasound beam beyond the occipital bone itself can yield a spuriously wide measurement. Also it is important not to include the occipital bone in the measurement. The quantitative description of this sign has been established only for fetuses between 15 and 19 weeks' gestation and should not be extended to older fetuses until adequate studies have been done on fetuses in a specific age range.

The presence of the abnormally wide nuchal skin fold in second-trimester fetuses has made this simple measurement valuable for the sonographic detection of some fetuses with Down syndrome. The majority of Down syndrome babies are born to women who are under 35 years of age and who are not routinely candidates for amniocentesis. With the increasing use of ultrasound for a variety of indications, this sign can be very valuable for detecting candidates who would not ordinarily be offered amniocentesis but who may in fact be at high risk for having a fetus with trisomy 21.

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First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions

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The frequency of first-trimester vaginal bleeding among 670 women with chromosomally normal fetal losses and 219 women with chromosomally abnormal losses was compared with that among 3089 women delivered at term. Vaginal bleeding early in gestation was predictive of pregnancy outcome in that moderate or heavy bleeding was associated with a fourfold risk of the loss of either chromosomally normal or abnormal conceptions. Spotting or slight bleeding was associated with a 2.7-fold risk of the loss of a chromosomally normal conception but was not associated with the loss of chromosomally abnormal conceptions when all abnormalities were categorized together. (Am J Obstet Gynecol 1987;157:1150-4.)

Key words: Vaginal bleeding, fetal loss, fetal karyotype

Vaginal bleeding, especially spotting or light bleeding, is a relatively common occurrence among women whose pregnancies proceed to term. Estimates of the frequency range from 15% to 17% when bleeding histories are obtained before delivery[1-3] or through in-person interviews after delivery.[4]

Fetal loss is almost always preceded by vaginal bleeding. There is usually bleeding before the expulsion of the products of conception but it can also occur early in gestation or intermittently throughout the pregnancy. Rates of fetal loss among women hospitalized for threatened abortion range from 35% to 50%.[5-8] However, hospitalized populations are not representative of women who experience light bleeding in the first trimester. Many cases of threatened abortion may actually be nonviable pregnancies at the time of admission. Since most fetal losses occur within 1 week of hospital admission,[6, 8] the bleeding in these cases may actually be the beginning of the expulsion process of a nonviable fetus rather than a predictor of the death of a viable fetus later in pregnancy.

Since bleeding occurs in pregnancies that end in fetal loss as well as those that proceed to term, the question remains as to whether the presence of bleeding early in pregnancy can predict subsequent fetal loss. The purpose of the analyses presented below is to determine if first-trimester vaginal bleeding that does not result in a spontaneous abortion at the time can predict fetal loss in the second trimester or later. A distinction is made between the loss of chromosomally normal and abnormal conceptions because chromosomally normal conceptions are more likely to be viable than chromosomally abnormal ones, most of which are never seen among term births.[9]

Material and methods

The data were derived from a case-control study of fetal loss carried out in New York City from 1975 through 1985 in three phases. The total case series consisted of 7872 women with a diagnosed fetal loss at <28 weeks' gestation. A total of 2346 women with fetal deaths occurring after 14 weeks' gestation were included in this analysis. Fourteen weeks was selected as a beginning point to allow for a minimum of 2 weeks to have lapsed between the onset of bleeding in the first 12 weeks of pregnancy and the fetal death.

An extensive in-person interview was administered to all women agreeing to participate. Of the 2346 eligible women, 1945 (83%) were interviewed. The questionnaires differed somewhat between the three phases but data were gathered throughout the study on the woman's obstetric, social, and medical history as well as events and exposures occurring in the current pregnancy. The vaginal bleeding histories in all three phases included date of onset, number of episodes, length of the first episode, and severity of the bleeding. The severity of the bleeding was categorized as spotting, slight, moderate, or heavy according to the woman's description of what the first episode of bleeding was like at its worst.

The products of conception were set up in tissue culture and a fetal karyotype was obtained for 979

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Table I. Frequency (percent) of first-trimester vaginal bleeding by sociodemographic characteristics among controls (women delivered at ≥28 weeks' gestation), New York City, 1975 to 1985

Characteristic	Public patients		Private patients	
	%	n	%	n
Maternal age (yr)				
≤20	10.3	458	13.3	15
21-25	12.6	722	18.3	104
26-30	18.6	560	20.5	292
31-35	22.6	349	23.7	249
≥36	18.8	218	21.3	122
Gravidity				
1	11.1	405	21.4	220
2	12.8	588	22.2	252
3	15.8	525	18.8	160
4	17.8	342	26.7	86
5	22.3	202	9.4	32
≥6	21.6	245	20.0	30
Previous spontaneous abortions				
Primigravid	11.1	405	21.4	220
0	15.6	1437	18.7	391
1	19.9	336	26.4	121
≥2	19.3	114	26.1	46
Cigarette smoking				
Nonsmoker	16.0	1721	21.3	694
Current smoker	14.7	565	19.5	87
Place of birth				
U.S.	15.0	1016	23.2	568
Foreign-born	16.2	1267	15.9	214
Maternal race				
White	15.0	300	23.0	592
Black	15.6	812	16.7	78
Hispanic	16.4	986	12.7	63
Other	13.4	209	18.4	49
Maternal education				
Less than high school	15.0	1117	11.1	27
High school graduate	15.6	672	15.3	124
More than high school	17.0	487	22.8	631
Phase of study entrance				
1	12.8	882	20.1	349
2	17.3	1034	20.6	310
3	17.9	391	26.0	123

(50%) of the interviewed women. The second interviews of 64 women who had entered the study more than once and 26 women with incomplete bleeding histories were excluded from the sample. Thus the final sample numbered 889 cases in which a fetal karyotype was determined, 670 of which were chromosomally normal and 219 of which were chromosomally abnormal. Abnormal karyotypes included trisomy, monosomy X, triploidy, tetraploidy, and other structural chromosomal abnormalities.

The control group was selected from the prenatal facilities of the medical centers. Control selection took place throughout the 10 years of the study but was not continuous over that time so that the total control population was smaller than the total case population. Controls were selected to be similar to the cases with respect to socioeconomic status (public or private hospital facility use) and maternal age. An additional criterion for a woman to be a control was that she had to be delivered of an infant at 28 weeks' gestation or later. Most controls were approached and interviewed at a prenatal visit in the second trimester of pregnancy. Controls interviewed before 14 weeks' gestation were excluded to ensure comparable ascertainment of first-trimester bleeding for cases and controls.

Table II. Frequency of first-trimester vaginal bleeding among cases (women with fetal losses ≥14 weeks' gestation) and controls (women delivered at ≥28 weeks' gestation)

	Public patients		Private patients	
	%	n	%	n
Cases				
Normal karyotype	30.5	492	41.0	178
Abnormal karyotype	25.7	144	33.3	75
Controls	15.7	2307	21.2	782

Of the 4205 controls identified, 3676 (87%) were interviewed. The second interviews of 63 women who had entered the study more than once were excluded. Of the remaining 3613 women, 3120 (86%) were interviewed after 14 weeks and 3089 of these women had complete bleeding histories.

The characteristics of first-trimester bleeding in each of the two case groups (normal and abnormal fetal karyotypes) were compared with those of controls in separate, conditional, maximum likelihood logistic regression analyses in which cases and controls were stratified by potentially confounding variables.[10, 11] The resulting adjusted odds ratios compared the presence of first-trimester bleeding in the cases with that in the controls.

The frequency of first-trimester bleeding by various sociodemographic characteristics of the control women was examined to identify potentially confounding variables (Table I). In both public and private payment groups, the frequency of bleeding increased with maternal age and a history of previous spontaneous abortion. There was an increase in the frequency of bleeding by gravidity among public, but not private, patients. There were no consistent differences by other maternal sociodemographic characteristics, cigarette smoking, or phase of study entrance.

Results

In both public and private payment groups, the rates of first-trimester bleeding were greatest among wom-

Table III. Adjusted* odds ratios and 95% confidence intervals relating first-trimester vaginal bleeding to fetal loss, defined by fetal karyotype (with controls as the reference group)

	Normal karyotype		*Abnormal karyotype*	
Characteristic of bleeding	*Odds ratio*	*95% Confidence interval*	*Odds ratio*	*95% Confidence interval*
Severity				
Spotting or slight	2.7	2.0-3.6	1.1	0.7-1.9
Moderate or heavy	3.6	2.1-6.2	4.9	2.1-11.6
Duration of first episode				
<1 wk	3.2	2.3-4.2	1.6	0.9-2.7
≥1 wk	3.7	2.1-6.6	8.0	2.7-23.1
No. of episodes				
1	2.1	1.6-2.8	1.1	0.6-1.8
>1	4.1	2.4-7.2	5.7	1.9-17.3

*Adjusted for maternal age, payment status, phase of study entrance, gestation (length of gestation at fetal loss for cases and at interview for controls), and previous spontaneous abortion.

Table IV. Odds ratios and 95% confidence intervals relating first-trimester bleeding to fetal loss by severity of bleeding and length of gestation*

		Spotting/slight		*Moderate/heavy*	
Gestation (wk)	*n*	*Odds ratio*	*95% Confidence intervals*	*Odds ratio*	*95% Confidence intervals*
14-15					
Normal karyotype	146	3.0	1.9-4.7	7.6	3.2-18.5
Abnormal karyotype	102	1.5	0.8-2.6	6.8	2.7-17.2
Controls	388				
16-17					
Normal karyotype	122	2.3	1.5-3.7	3.1	1.4-7.0
Abnormal karyotype	56	1.6	0.8-3.2	2.4	0.8-7.5
Controls	500				
18-19					
Normal karyotype	99	2.5	1.5-4.3	3.2	1.2-8.8
Abnormal karyotype	19	3.0	1.0-8.8	9.9	2.5-39.7
Controls	556				
20-21					
Normal karyotype	129	1.3	0.8-2.2	3.6	1.6-8.0
Abnormal karyotype	16	0.8	0.2-3.7	4.6	0.9-22.4
Controls	514				
22-23					
Normal karyotype	92	2.7	1.6-4.8	1.0	0.2-4.9
Abnormal karyotype	12	0.6	0.1-4.9	—†	
Controls	349				
≥24					
Normal karyotype	82	1.5	0.8-2.8	2.0	0.8-5.5
Abnormal karyotype	14	0.5	0.1-4.1	2.1	0.3-16.7
Controls	782				

*Length of gestation at fetal loss (cases) and at interview (controls).
†Not calculated because of empty cells.

en aborting chromosomally normal conceptions but were also increased among women aborting chromosomally abnormal conceptions when compared with rates among controls (Table II). The intensity of first-trimester bleeding was further characterized by its severity, the duration of the first episode, and the number of episodes beginning in the first trimester. Table III shows the odds ratios relating each of these characteristics of bleeding to chromosomally normal and abnormal fetal losses. These were adjusted for maternal age, phase of study entrance, number of previous spontaneous abortions, and gestation.

Both the chromosomally normal and abnormal case groups were more likely than controls to experience bleeding that was moderate or heavy, that was longer than 1 week's duration, and that consisted of multiple episodes, with odds ratios ranging from 3.6 to 4.1 for the chromosomally normal losses and from 4.9 to 8.0

for the chromosomally abnormal losses. Less intense bleeding, that is, spotting or slight, lasting <1 week, or consisting of only one episode, was more common among women with chromosomally normal losses than among controls, with odds ratios ranging from 2.1 to 3.2. There was no difference between women with chromosomally abnormal losses and controls with respect to light bleeding; odds ratios ranged from 1.1 to 1.6.

The observed associations between bleeding of either intensity and fetal loss did not vary with maternal age, reproductive history, payment status, or phase of study entrance. However, the association of moderate or heavy bleeding with both normal and abnormal fetal loss did vary by week of gestation (Table IV). The association is greatest for those losses occurring at 14 to 15 weeks and decreases thereafter. This trend is more apparent for the chromosomally normal conceptions than for the abnormal conceptions, although the odds ratios for abnormal conceptions are based on small samples in later gestations.

The association between spotting or slight bleeding and chromosomally normal loss remained relatively constant with the duration of gestation of the fetal loss. The odds ratios for chromosomally normal losses at any length of gestation did not vary greatly from the overall odds ratio of 2.7. For chromosomally abnormal losses, the odds ratios did not vary significantly from 1.0 at any length of gestation.

When a conceptus has a chromosomal abnormality, its survival is severely compromised. However, some abnormalities are compatible with survival and are seen at term among stillbirths or live births whereas others are always lethal and the fetuses never survive to term. Table V shows the odds ratios for spotting or slight first-trimester bleeding by specific groups of chromosomal abnormalities. Although the same sizes are small, there may be some variation in risk between abnormalities, raised odds ratios for triploid (odds ratio = 2.3) and trisomy 21 (odds ratio = 3.0) losses, and no association for nonviable trisomies (odds ratio = 1.1) and monosomy X (odds ratio = 0.6) losses.

Table V. Odds ratios and 95% confidence intervals relating first-trimester spotting or slight bleeding to specific chromosomal abnormalities (with controls as the reference group)

Abnormality	*n**	*Odds ratio*	*95% Confidence interval*
Viable trisomies			
21	15	3.0	1.0-8.9
13, 18, X	9	1.8	0.3-9.8
Nonviable trisomies	48	1.1	0.5-2.5
45, XO	30	0.6	0.2-2.1
Triploidy	56	2.3	1.2-4.3

*n = Number in each abnormal case group, excluding women with moderate or heavy first-trimester bleeding.

Comment

The findings of this study suggest that the presence and type of first-trimester vaginal bleeding can predict, to some extent, the outcome of the pregnancy and the characteristics of the conceptus. Moderate or heavy bleeding in the first trimester was associated with both chromosomally normal and abnormal losses in the second trimester. Even though the bleeding preceded the diagnosis of fetal loss by several weeks, it is possible that in some of these cases the conceptus was not viable at the onset of the bleeding. Ultrasound examinations were not routinely carried out to document fetal viability, especially in the early years of the study. If they had been, undoubtedly some of the first-trimester bleeding episodes would have been diagnosed as fetal deaths and the pregnancies would have been terminated. Thus our odds ratios, especially those pertaining to moderate or heavy bleeding and its relationship to fetal loss early in the second trimester, could be inflated because they include pregnancies that may not have been viable at the time of the bleeding.

Fetal viability at the time of the bleeding is less of an issue for spotting or slight bleeding since this type of bleeding is associated with a constant, increased risk of chromosomally normal loss at all lengths of gestation. Chromosomally normal conceptions have a greater potential for viability than abnormal conceptions and it is unlikely that a conceptus that was nonviable at 12 weeks would not abort until the end of the second trimester. It appears therefore that spotting or slight bleeding occurs when the conceptus is still viable, although at an increased risk of death later in pregnancy.

Light bleeding may also be increased in those women aborting conceptions with trisomy 21, a chromosomal abnormality that is compatible with survival. Although triploid conceptions never survive the perinatal period, they do occasionally come to term and, along with trisomy 21, are more likely than other chromosomally abnormal conceptions to abort late in gestation.[12]

In conclusion, the pregnancies of women who experience moderate or heavy bleeding in the first trimester are likely to end in fetal loss sometime during gestation. Spotting or slight bleeding in the first trimester indicates that a woman is at a moderately increased risk, about 2.7 times that of a woman with no bleeding, of losing a chromosomally normal conception. Additionally, it is possible that those women with chromosomally abnormal conceptions that tend to be lost late in gestation also have bleeding characteristics similar to those of women losing chromosomally normal conceptions.

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Emotional responses of pregnant women to chorionic villi sampling or amniocentesis

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Seventy-four "high-risk" pregnant women interested in participating in a clinical trial comparing chorionic villi sampling and amniocentesis were initially assessed on five background measures and for anxiety, depression, hostility, and concern about abortion. The 61 women who then agreed to be randomized into the chorionic villi sampling and amniocentesis groups were assessed three additional times between 9 and 22 weeks' gestational age. Physical discomfort experienced during the diagnostic procedure was also assessed. Women in the chorionic villi sampling group underwent a reduction in anxiety up to 10 weeks earlier and reported less procedure-related discomfort than women in the amniocentesis group. The group × time interaction for depression was also significant, with women undergoing chorionic villi sampling reporting an earlier decrease in depression than women undergoing amniocentesis. No differences were found between the two groups in hostility or concern about abortion. Findings were discussed in the context of evidence linking prenatal maternal emotionality to an increased risk of obstetric complications. (AM J OBSTET GYNECOL 1987;157:1155-60.)

Key Words: Prenatal diagnosis, amniocentesis, chorionic villi sampling, maternal emotionality

Until the comparatively recent development and use of chorionic villi sampling (CVS) as a first-trimester procedure, genetic diagnosis was not possible before the midtrimester of pregnancy.[1] Amniocentesis has been the most commonly used midtrimester genetic diagnostic technique, and although the physical risks associated with amniocentesis are comparatively low (<0.5%),[2,3] the emotional distress associated with midtrimester genetic diagnosis is widely acknowledged, both in the research literature[4-6] and in anecdotal reports.[7] By comparison, the physical risks of CVS were initially reported to be greater (3% to 5%),[8] although more recent data suggest a more comparable procedure-induced loss rate in the range of 1% to 2%.[9] A direct comparison of physical risk rates between CVS and amniocentesis is complicated by (1) the higher baseline rate of spontaneous abortion during the first trimester than during the midtrimester and (2) imprecise figures for the rate of spontaneous abortion during the first trimester. Reported risk rates for CVS generally include the background rate of spontaneous abortion after that procedure, while reported risk rates for amniocentesis typically do not.

Despite the apparently greater physical risk at the time the present study was conducted, it was felt that CVS may arouse less emotional distress than amniocentesis for at least three reasons. First, the sampling occurs as early as 9 weeks' gestation, 7 weeks earlier than with amniocentesis, obviating a lengthy pretest waiting period that could arouse considerable emotional distress. Second, results after CVS are available within 1 week and in some centers within 48 hours. Results with amniocentesis usually require 3½ to 4 weeks, again imposing a longer and potentially distressing posttest waiting period. Third, elective termination can occur up to 10 weeks earlier in pregnancy after CVS than after amniocentesis. This has obvious benefits to the patient both in terms of the type of procedure (dilation and curettage versus prostaglandin injection) and the woman's personal commitment to the pregnancy and attachment to her fetus. For these reasons, emotional distress directly associated with CVS should be less than that associated with amniocentesis. Respondents in a recent study[10] confirmed that these features could enhance the acceptability of CVS for prenatal diagnosis, particularly if the risk rate was found to be equivalent to that of amniocentesis. However, this was a hypothetical situation, as none of the women in the study had actually undergone CVS. Empirical data available from the current research, although not reported here, indicate that women who underwent either CVS or amniocentesis chose CVS

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when asked to project the use of genetic diagnosis to future pregnancies.[11]

Because women referred for prenatal diagnoses have high-risk pregnancies, the emotional distress evoked by these procedures may interact with and exacerbate the already elevated level of risk. There is considerable research that has implicated self-reported anxiety during pregnancy to subsequent obstetric complications,[12, 13] including somatic complaints during pregnancy[14] and labor and delivery problems.[15, 16] Hence the prenatal diagnostic procedure that evokes the least maternal emotionality for the shortest period would be the more desirable, because of a correspondingly lower potential to contribute to subsequent obstetric complications. It was expected that CVS would evoke less negative maternal emotionality than amniocentesis because it involves an earlier administration, a shorter posttest waiting period, and a less traumatic elective termination of pregnancy. The present study is an initial attempt to validate this hypothesis.

Material and methods

Seventy-four women with high-risk pregnancies of less than 11 to 12 weeks' gestational age who had been referred for prenatal diagnoses and who expressed an interest in the medical trial comparing CVS and amniocentesis were asked to participate. All were residents of British Columbia whose medical expenses were covered by the Government health-care plan, and all prenatal genetic diagnoses were conducted at Grace Hospital in Vancouver, British Columbia. Sixty-one women who agreed to continue in the study after an intake counseling session were randomized into either the CVS group (29 women) or the amniocentesis group (32 women), and dates for their prenatal tests were set at this time. All women in the study satisfied several inclusion criteria for prenatal genetic diagnosis and subject assignment was entirely random. No group assignment was made according to categories of risk. These women were asked to complete four separate sets of questionnaries during their pregnancies, although four of these women experienced spontaneous abortions and completed only the initial set. The 13 women who declined to take part in the medical trial following the intake session (nonparticipation group) completed only the initial set of questionnaires and were compared on these measures with the 61 women who agreed to participate in the trial.

Description of measures. The selection of instruments was dictated in part by the limited time available to respondents while at the hospital.

1. Background information form. This form solicited information regarding age, parity, fetal loss (a composite measure of miscarriages, stillbirths, and induced abortions), annual family income, and living arrangement of the mother and her partner.
2. Multiple Affect Adjective Checklist (MAACL). The MAACL-general is a 132-adjective checklist that measures anxiety, hostility, and depression and has been validated by a variety of settings and subjects.[17] The MAACL is psychometrically sound and correlates well with other measures of these variables, yet it can be completed by most subjects in only 5 minutes.
3. Procedure Discomfort Scale (PDS). The PDS was devised for the present investigation after a brief pilot study and was designed to compare the physical discomfort experienced by women undergoing CVS or amniocentesis. It is a Likert-type rating scale[18] from 1 to 15 that asks the respondent to rate the discomfort she experienced during her prenatal diagnostic procedure.
4. Concern about Abortion Questionnaire (CAQ). The CAQ is a form devised to compare the emotional reactions to abortion of women undergoing CVS or amniocentesis, on the basis of similar instruments that have been used at Grace Hospital to assess the psychological effects of prenatal ultrasound scanning.[19] This form asked the subject to imagine a situation in which she has learned that her fetus has a serious genetic disorder for which elective abortion may be appropriate.

Other instruments assessed the impact of prenatal diagnosis on commitment to the pregnancy. The acceptibility of CVS versus amniocentesis was also assessed among women who had undergone one of these procedures and who had knowledge of the other. These data will be presented in a subsequent report.

Procedure. After providing informed consent, the 74 subjects were asked to complete four sets of questionnaires, each requiring from 10 to 15 minutes. Three of the sets were administered at equivalent measurement points for both groups on the basis of the subjects' gestational ages, and the fourth set was administered immediately after either the CVS or the amniocentesis procedure. The repeated administrations were necessary to compare the course of maternal emotionality for women in both groups over the period of the study.

The first set (background information, MAACL, CAQ) was administered at the hospital immediately before the intake counseling session, at time 1, which occurred at 8 to 11 weeks' gestation. Thirteen subjects declined further participation in the trial and completed no further questionnaires. These women were designated as the nonparticipation group.

A second set of questionnaires (MAACL) was administered by mail at time 2, at approximately 13 to 14 weeks' gestation for women in both groups. The CVS

Table I. Means for anxiety, depression, hostility, concern about abortion, and procedure discomfort: CVS group

	Time 1 (8-11 wk)	*Time of procedure (9-12 wk)*	*Time 2 (13-14 wk)*	*Time 3 (22 wk)*
Anxiety	9.43	9.32	5.54*	4.79
Depression	14.25	13.79	12.39	11.14†
Hostility	8.86	8.82	8.36	7.71
Concern about abortion	53.28	51.79	—	—
Procedure discomfort	—	4.04	—	—

*Change from preceding measurement, $p < 0.01$.
†Change from preceding measurement, $p < 0.05$.

Table II. Means for anxiety, depression, hostility, concern about abortion, and procedure discomfort: Amniocentesis group

	Time 1 (8-11 wk)	*Time 2 (13-14 wk)*	*Time of procedure (16-17 wk)*	*Time 3 (22 wk)*
Anxiety	9.31	9.21	9.14	5.26*
Depression	13.66	14.21	14.34	10.55*
Hostility	8.93	9.48	9.83	8.34
Concern about abortion	53.60	—	54.35	—
Procedure discomfort	—	—	7.41	—

*Change from preceding measurement, $p < 0.01$.

group had received their results,* while the amniocentesis group were still awaiting their procedures.

A third set of questionnaires (MAACL) was administered by mail at time 3, at approximately 22 weeks' gestation for women in both groups. All women in the trial had received final notification of their results.

A fourth set of questionnaires (MAACL, CAQ, PDS) was administered in the hospital at the time of the procedure for women in both groups. The CVS group completed these forms at 9 to 12 weeks' gestation, while the amniocentesis group completed these forms at 16 to 17 weeks' gestation. This administration was chronologically the second administration for the CVS group and the third administration for the amniocentesis group.

Results

The data were submitted to analyses of variance, and pairwise comparisons were conducted where appropriate. This section first reports on background variables; then findings for each of the five dependent measures are reported sequentially for the CVS and amniocentesis groups. Additional analyses comparing the CVS, amniocentesis, and nonparticipation groups on all measures were conducted at time 1 (intake). As expected, none of the main effects for group was significant at time 1.

Background variables. Results from the five background variables of age, number of children, number of fetal losses, family income, and living arrangement revealed no significant differences among the three groups.

Anxiety. Means for anxiety at each of the four measurement points are provided in Tables I and II for the CVS and amniocentesis groups, respectively. Table I reveals that the CVS groups underwent a significant reduction in anxiety from the time of procedure to time 2 (9 to 14 weeks' gestation), $F(1,27) = 13.95$, $p < 0.001$. Table II shows that the amniocentesis group did not undergo a corresponding decrease in anxiety until later, from the time of procedure to time 3 (16 to 22 weeks' gestation), $F(1,28) = 41.57$, $p < 0.001$.

Comparisons between these two groups at the three comparable measurement points confirm a significant overall difference in the course of anxiety between the two groups. The group × time interaction for anxiety was significant, $F(2,110) = 9.11$, $p < 0.001$, and Fig. 1 shows that the CVS group reported significantly less anxiety at time 2, $F(1,55) = 17.28$, $p < 0.001$.

These results clearly show that both groups of women underwent a reduction in anxiety after prenatal diagnosis, with the CVS group reporting the change as much as 8 weeks earlier than the amniocentesis group.

Depression. Tables I and II provide means for

*Data from the one woman in the study who received abnormal results were incomplete and were not included in the analyses.

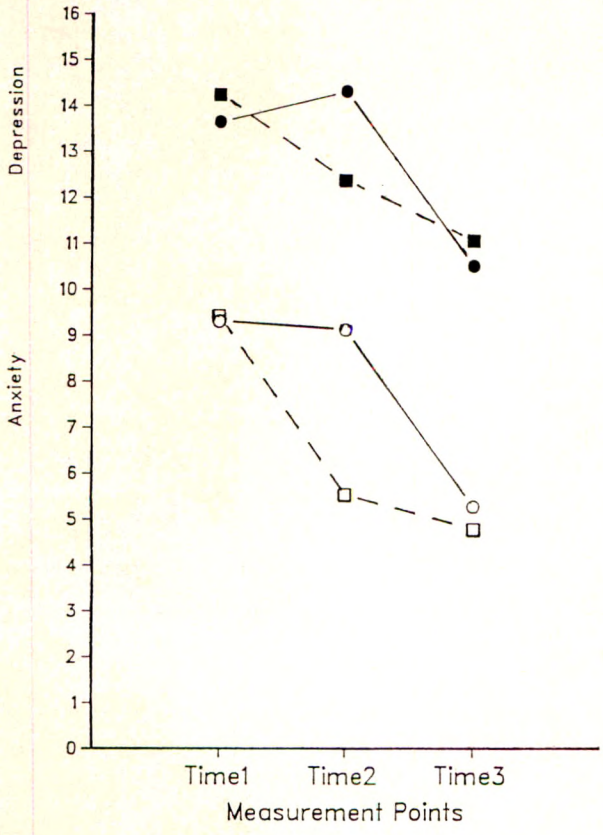


Fig. 1. Changes in anxiety and depression across measurement points for women undergoing either amniocentesis or chorionic villi sampling. ○, Amniocentesis: anxiety. □, Chorionic villi sampling: anxiety. ●, Amniocentesis: depression. ■, Chorionic villi sampling: depression.

depression at each of the four measurement points for the CVS and amniocentesis groups. The CVS group underwent its largest decrease in depression from time 2 to time 3, $F(1,27) = 5.20$, $p = 0.03$. The amniocentesis group underwent a significant decrease in depression from the time of procedure to time 3, $F(1,28) = 23.35$, $p < 0.001$.

Comparisons between the two groups at time 1, time 2, and time 3 are shown in Fig. 1. The group × time interaction was significant, $F(2,110) = 4.71$, $p < 0.01$. Again, the greatest difference between the two groups emerged at time 2, suggesting that women undergoing CVS experience a decline in depressive feelings 8 weeks earlier than women undergoing amniocentesis, but this difference falls just short of significance, $F(1,55) = 3.13$, $p = 0.08$.

Hostility. Means for hostility at the four measurement points for both groups are shown in Tables I and II. None of the changes over adjacent measurement points reached significance for either group. Comparisons between the two groups at time 1, time 2, and time 3 revealed no significance in the simple main effects or in the group × time interaction. These results suggest that feelings of hostility among subject women were unrelated to diagnostic intervention.

Procedure discomfort. Means for procedure discomfort, assessed immediately after the CVS or amniocentesis procedure, are shown in Tables I and II. The CVS group reported significantly less physical discomfort than the amniocentesis group, $F(1,55) = 14.42$, $p < 0.001$.

Concern about abortion. Tables I and II provide means for concern about abortion for the two measurement points in each of the two participating groups. No significant change over time was observed for either group, nor were the differences between the two groups significant at time 1 or the time of procedure. Because concern about abortion remained uniformly high for women in both groups before notification of diagnostic outcome, this questionnaire may have sensitized women to the stress of a potential abortion. Observations during data collection support this interpretation.

Comment

The present study indicates that emotional responses of pregnant women undergoing prenatal genetic diagnoses can vary according to the type of procedure experienced. Significant changes in reported anxiety were observed across the four measurement points for both the CVS and the amniocentesis groups. Of particular interest was the finding that the CVS women underwent a significant reduction in anxiety after prenatal testing, presumably after notification of the test results at 10 to 13 weeks. By comparison, the anxiety level among women in the amniocentesis group remained relatively high throughout the study, declining after notification of the test results at 19 to 20 weeks. Previous research into prenatal diagnosis has shown that anxiety does not abate until after the test results have been received and women are reassured that no abnormalities have been detected.[4, 20]

If the basis of the anxiety reduction is the notification of test results, women undergoing CVS will experience a decline in anxiety approximately 8 weeks earlier than women having amniocentesis. The potential importance of this difference is underscored by earlier studies that have characterized the first trimester of pregnancy as one of physical upset, emotional upheaval, and pronounced mood swings, as the pregnant women makes the initial adjustment to the change in her life.[21, 22] By contrast, the midtrimester is described as one of relative quiescence and stability, as physical symptoms subside, anxiety declines, and the initial adjustment process concludes. CVS would likely add to the emotional tumult during the first trimester, but the extent to which the increase in anxiety associated with the procedure would be detrimental is difficult to determine. The increased

stress of CVS may be offset by the early attention given the pregnancy, such as the viewing of the fetus through ultrasound.[23, 24] The fact that the entire process of first contact, prenatal testing, and notification of results can be completed within a 2-week span suggests that any CVS-induced increment in anxiety over the background level common during the first trimester may be minimal.

Women scheduled for amniocentesis typically find themselves anticipating the procedure with some apprehension. The present findings that the long waiting period before amniocentesis and subsequently for the results is one of sustained outcome apprehension are supported by anecdotal reports that this period, which extends to 20 weeks' gestation, is one of concern and agitation.[7] This period of sustained anxiety is especially disturbing in view of the finding that it is the anxiety experienced before the fifth month of pregnancy that is most predictive of obstetric complications.[25] Anxiety among women undergoing CVS would have declined by the third month, while anxiety associated with outcome apprehension among women undergoing amniocentesis would persist until the fifth month, precisely the period of the highest risk. Given that prenatal diagnostic procedures arouse anxiety, the current research indicates that CVS arouses less anxiety over a shorter period of time. In view of the research showing that significant differences in anxiety exist between complicated and uncomplicated pregnancies and that anxiety may predispose expectant mothers to obstetric complications,[12, 16, 25] the present findings that show CVS to arouse less anxiety over the long term may be of clinical importance.

The findings for reported feelings of depression among women in both groups tend to parallel those of anxiety, providing further support for the psychological desirability of CVS. The relationship between prenatal depression and obstetric outcome has not been widely investigated,[13] and the present results must be interpreted with some caution. The significant difference in reported physical discomfort associated with each procedure is of considerable interest. Clearly women undergoing CVS report far less physical discomfort that women having amniocentesis. This factor alone might be expected over time to contribute to a preference for CVS among women requiring genetic diagnosis.

Given that pregnancy is a period of psychological upheaval, particularly when there is an associated risk factor, the reduction of anxiety is of considerable value. However, prenatal diagnosis, while an important element of the technology of modern obstetric care, may have the impact of increasing the stress and anxiety that women experience. This research has demonstrated that CVS, in comparison with amniocentesis, is clearly the procedure of choice with regard to the psychological impact of genetic diagnosis in pregnancy. The implication is that as the risk rates attendant with CVS are stabilized, the desirability of the procedure should undergo a corresponding increase relative to amniocentesis.

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Hereditary protein C deficiency during pregnancy

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Hereditary protein C, a primary hypercoagulable state, has not been previously associated with preeclamptic toxemia. A woman with previous preeclamptic toxemia and fetal deaths presented with recurrent deep vein thrombosis during her third pregnancy. Hereditary protein C was diagnosed but full heparinization followed by low-dose heparin failed to prevent preeclamptic toxemia. (Am J Obstet Gynecol 1987;157:1160-1.)

Key words: Protein C, toxemia, thrombosis

Protein C, a vitamin K–dependent plasma protein, functions in its activated form as a natural anticoagulant by inactivation of factors Va and VIIIa and by stimulation of fibrinolysis. Conversion of protein C to activated protein C is induced by a complex of thrombin and the endothelial cell receptor thrombomodulin. The function of activated protein C is enhanced by a cofactor, protein S. Recurrent thromboembolic episodes are observed in young patients with hereditary protein C deficiency. We describe here a pregnant patient with hereditary protein C deficiency who presented with recurrent deep vein thrombosis and preeclamptic toxemia.

Case report

A 22-year old woman first presented with severe preeclamptic toxemia at 26 weeks' gestation that necessitated termination of pregnancy by cesarean section. The neonate died at 10 days of hyaline membrane disease. Her second pregnancy developed normally until the thirty-sixth week when an intrauterine fetal death occurred. A repeat cesarean section was performed, during which dehiscence of the scar was noted. Autopsy revealed no fetal abnormality but extensive infarctions and calcifications were found in the placenta. Three weeks after her discharge, the patient was readmitted because of pseudotumor cerebri, which responded to treatment with dexamethasone. One month later, thrombosis of the right femoral vein was observed. Intravenous heparin was administered for 10 days followed by sodium warfarin daily for 4 months. Two months after discontinuation of therapy the patient again became pregnant and at 8 weeks' gestation another episode of thrombosis of the right femoral vein occurred. Intravenous heparin, 40,000 U/day, was given but on the eighth day thrombosis of the left femoral vein was observed. At this stage a primary hypercoagulable state was considered and explored. Antithrombin III activity (80 U/dl), protein S antigen (139 U/dl), factor VII activity (70 U/dl), and factor II activity (140 U/dl) were all within normal limits. However, protein C antigen was 55 U/dl (normal range, 68 to 130 U/dl). A family study revealed that the patient's father and three siblings also had protein C deficiency (Fig. 1). They were all asymptomatic. With continuous heparinization the acute thrombotic episodes subsided and the patient was placed on a regimen of subcutaneous heparin, 7500 U twice daily. At 34 weeks signs of preeclamptic toxemia developed: blood pressure of 160/100 mm Hg, edema, and proteinuria. Ultrasound imaging revealed moderate intrauterine growth retardation. A third cesarean section was performed at 35 weeks and a female infant weighing 1750 gm, with an Apgar score of 10 at 1 minute, was delivered. Neonatal evidence of intrauterine growth retardation was ob-

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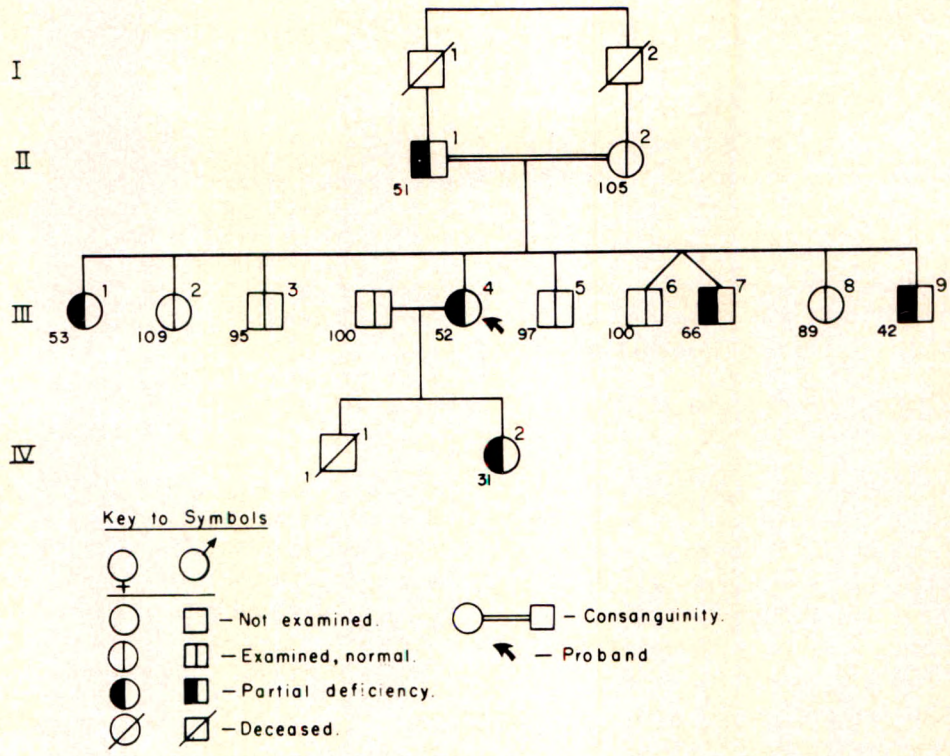


Fig. 1. Pedigree of family with members affected by hereditary protein C deficiency. *Numbers* below symbols are the observed protein C levels (units per deciliter). Note that the father and three siblings of the proband have protein C deficiency. The low level of protein C in the patient's daughter is probably related to her newborn status at examination.

served. The placenta showed extensive areas of infarction and calcification. The postoperative course was uneventful and the patient was discharged on a lifetime regimen of warfarin therapy.

Comment

Our study illustrates that patients with a hereditary hypercoagulable state are at risk of thrombosis during pregnancy. This was previously documented in patients with antithrombin III deficiency.[1] Unexplained or first-trimester thromboembolism should therefore alert the physician to examine the levels of antithrombin III, protein C, and protein S. Once the diagnosis of a hereditary hypercoagulable state is made during pregnancy or when a patient known to be affected becomes pregnant, the question of management must be addressed. During the first trimester of pregnancy oral anticoagulants are potentially teratogenic and therefore contraindicated. Furthermore, since sodium warfarin traverses the placenta, there may be a risk of fetal bleeding throughout pregnancy. Consequently, we chose to treat our patient with low-dose subcutaneous heparin throughout pregnancy. With this treatment modality thromboembolism did not recur, yet preeclamptic toxemia did develop.

It is widely accepted that placental insufficiency caused by acute atherosis and thrombosis of placental vessels may lead to intrauterine growth retardation in preeclamptic toxemia. Gilabert et al.[2] recently reported an acquired protein C deficiency and an increase in protein C inhibitor levels in patients with severe preeclamptic toxemia. Hence it is not unlikely that our patient developed preeclamptic toxemia and placental insufficiency in three successive pregnancies because of hereditary protein C deficiency. However, further observations in pregnant patients with hereditary protein C deficiency are needed in order to establish the validity of this association.

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A very rare fetal malformation: The cutaneous widespread vascular hamartomatosis

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A rare fetal malformation with a large number of vascular masses over the entire body diagnosed by ultrasound examination at 24 weeks of gestation is described. No report of this anomaly has been found in the international literature. (Am J Obstet Gynecol 1987;157:1162-3.)

Key words: Fetal malformation, vascular cutaneous hamartomatosis

The fetal vascular malformations are numerous; the more simple ones enable a normal life, but the more complicated ones do not. A case of vascular malformation with several large vascular masses detected antenatally by ultrasound examination is reported; these masses were on the skin and in the upper section of the thoracic spinal cord. No report of this anomaly has been found in the international literature, so we presume this to be the only reported case.

Case report

C. M., an 18-year-old woman, gravida 2, para 0, with one spontaneous abortion, was examined at 24 weeks of amenorrhea. Past medical history was unremarkable. The woman had not been exposed to any known teratologic agent. In September 1984 outpatient ultrasonography revealed polyhydramnios and fetal anomalies. Level 2 ultrasonography pointed out a single living fetus with several echo-free subcutaneous-cutaneous masses deforming its profile; the biggest ones were localized on the thorax, two in the front (diameter about 10 cm) and three at the back (diameter about 8 cm). Edema of the scalp also was present (Fig. 1). On November 9, 1984, a cesarean section was performed because this malformation was incompatible with life and would not have enabled vaginal delivery. A male fetus weighing 1300 gm and 30 cm long was delivered and died after a few minutes (Fig. 2).

Autopsy examination confirmed the presence of cutaneous neoformations on the thorax, abdomen, back, and limbs. These masses were of varying width and morphologic features; the smallest were of a papillary appearance, and the larger ones were exuberant, each

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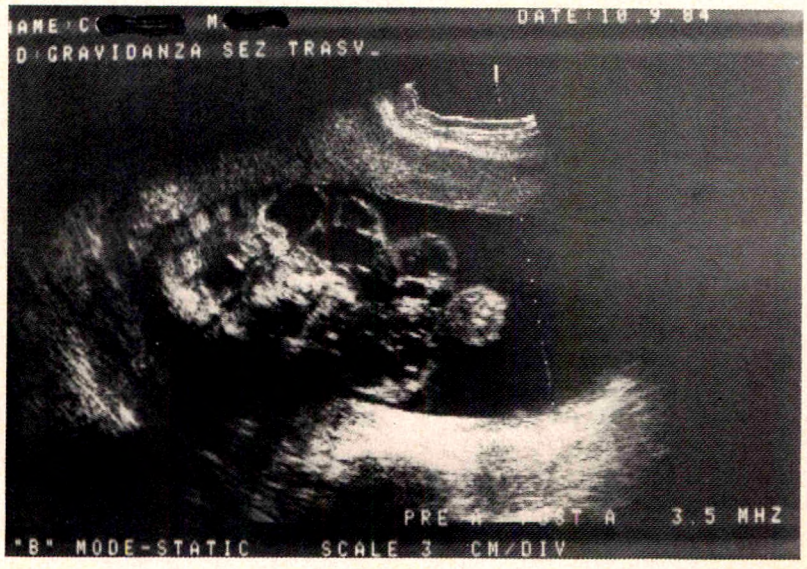


Fig. 1. Ultrasound appearance of the fetus. In this section one can see the large quantity of echo-free masses in the front and at the back.

one having a red wine–like color. The skin over the masses was taut and congested. The larger masses appeared multiloculated when cut, and each one contained bloody dark red liquid. There was one small mass in the upper region of the thoracic spinal cord. The internal organs were free of malformations. Radiologic study of the fetus did not reveal anomalies of the bone.

Microscopic study revealed that these masses were formed by capillary-like vessels with cavernous dilatation and congestion. The dermis and subcutaneous tissues were edematous with beginning sclerosis. The pathologic diagnosis was cutaneous widespread vascular hamartomatosis.

Comment

This case is unique because of the widespread cutaneous lesions with only one in a medullary location, the exuberance of the lesions, and the absence of associated malformations. Under the microscope these masses look like cavernous hemangiomas, but, unlike this condition, they are numerous, they are not associated with mesodermic dysplasia, like Maffucci's syndrome, and their location is not splanchnic, as in "blue rubber bleb nevus syndrome." Moreover, these masses differ from venous ectasia (nevus flammeus, nevus vinosus) and associated syndromes (Sturge-Weber syndrome and others) because they are characterized by proliferation of newly formed vessels instead of ectatic vessels. Finally, another pecularity of this case is the isolated involvement of a very small part of the spinal cord.

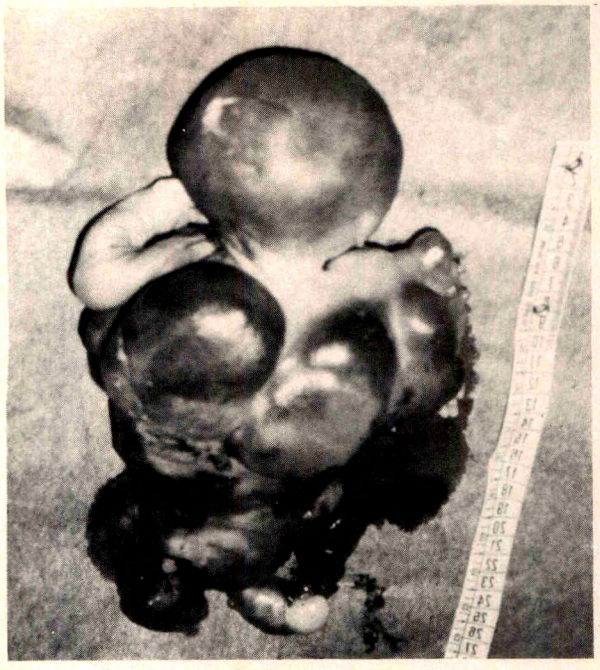


Fig. 2. Macroscopic appearance of the newborn infant.

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Updated pregnancy rates for the Today contraceptive sponge

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Pregnancy rates (method and user) were evaluated for 2245 women who participated in the phase III clinical trials of the Today contraceptive sponge. User and method pregnancy rates were similar for women of different ages and parities. The 1-year method and user pregnancy rates (life table per 100 women) were 8.2 and 5.6, respectively. (AM J OBSTET GYNECOL 1987;157:1164-5.)

Key words: Contraceptive sponge, pregnancy rates, parity

The Today contraceptive sponge (VLI Corporation, Irvine, California) first became available to consumers in the United States in 1983. Since that time it has been approved for use and is being sold in a number of other countries including Korea, Sweden, Switzerland, and the United Kingdom. Phase III clinical trials of the Today sponge were started in 1979, and since that time data on 2245 women have been accumulated. Pregnancy data from studies conducted in the United States, the United Kingdom, and Yugoslavia have been published previously[1-3] and account for only about 55% of the women included in the trials. This communication presents pregnancy rate data from the worldwide clinical trials of the contraceptive sponge and includes data from previously published studies.[1-3] Since the effectiveness of vaginal contraceptives depends on whether they are correctly and consistently used, pregnancy rates were evaluated according to whether they were a result of failure of the user to use the sponge as directed (user failure) or because the method failed even though the sponge was correctly used (method failure).

Methods and material

The Today sponge is made of proprietary polyurethane and contains 1 gm of the spermicide nonoxynol 9, as well as small amounts of preservatives (citric, sorbic, and benzoic acids) that help to maintain its pH at about 4 to 5. The sponge is 2.5 cm thick and has a diameter of 5.5 cm. About 125 mg of nonoxynol 9 is eluted from the sponge during a 24-hour use period. The sponge is inserted into the vagina at any time up to 24 hours before intercourse. Repeated intercourse may occur with the sponge in place. The sponge is left in place for at least 6 hours after intercourse. The maximum recommended use time is 24 hours. Use of the sponge during menstruation is not recommended.

Table I. Cumulative 12-month life-table method and user pregnancy rates (per 100 women)

	No. of women	*Pregnancy rate*	
		Method (mean ± SEM)	*User (mean ± SEM)*
All clinics	2042	8.2 ± 0.8	5.6 ± 0.7
Nulliparous women	1088	7.8 ± 1.0	5.3 ± 0.9
Multiparous women	954	8.7 ± 1.2	5.6 ± 1.0
Age (yr)			
<19	133	2.5 ± 1.4	4.5 ± 3.7
19-26	941	9.0 ± 1.3	6.2 ± 1.1
27-40	933	7.2 ± 1.1	5.0 ± 0.9
>40	35	—†	—†

*Women not followed up were excluded from the table.
†Too few women for calculation of reliable life-table rate.

Pregnancy rates are based on data gathered on 2245 women from 26 clinics (Bangladesh, two; Canada, one; Israel, one; Sweden, one; Switzerland, four; Taiwan, one; United Kingdom, one; United States, 13; Yugoslavia, two). No follow-up data or incomplete records were obtained for 9.0% of the subjects. All pregnancies were classified as either user or method failures on the basis of information in the case report forms and coital logs (completed by only a subset of subjects) and from comments provided by the women and their physicians. Insufficient information was provided to classify 13 (6.7%) of the 172 pregnancies (103 method, 69 user) occurring during the 12-month follow-up period. These pregnancies were classified arbitrarily and conservatively as method failures. Pregnancy rates were computed by use of life-table methods that provide estimates of the risk of pregnancy as a function of time. Pregnancy rates were compared by means of the log rank test statistic. All of the analyses are based on the pooled data from the clinics since there were too few subjects for evaluation in all but a few clinics.

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Results

The overall, cumulative 12-month pregnancy rate was 13.3 per 100 women (method, 8.2; user, 5.6). User and method pregnancy rates are given in Table I. Neither method nor user pregnancy rates depended on parity (nulliparous versus parous) or age of the women ($p > 0.10$). The same holds true for the combined pregnancy rate. At seven of the 13 clinics in the United States, 88 women volunteered to participate in the studies for a second year. The overall, cumulative pregnancy rate for these women during their second year of use was 3.4 per 100 women.

The lost-to-follow-up rates varied from clinic to clinic. However, there was no relationship between the pregnancy rates (user, method, overall) and lost-to-follow-up rates ($p > 0.10$). The cumulative 12-month pregnancy rates (user, method, overall) were not similar across clinics ($p < 0.05$).

Pregnancy rates (user, method, overall) based on the data from clinics that had women complete coital logs were similar ($p > 0.10$) for women who completed the coital logs and for those who did not. The principal use of these logs was to evaluate patterns of sponge use and provided no useful data for the analyses of pregnancy rates.

Comment

The presentation of method pregnancy rates is important so that women may assess their risk of pregnancy if they use the sponge consistently and correctly according to the user directions. Overall pregnancy rates do not provide this information.

The data presented in this paper include the results of previously published studies from the United States,[1] the United Kingdom,[2] and Yugoslavia.[3] The data from the study of 721 subjects conducted in the United States at 13 clinics[1] showed that the cumulative 12-month overall pregnancy rate was related to parity but not age. The report did not provide data on method or user pregnancy rates or on the pregnancy rates reported at the individual clinics. The study conducted in the United Kingdom[2] gave higher overall pregnancy rates than did the study in the United States but no significant differences in the overall pregnancy rates for parous and nulliparous sponge users were reported. No data on either method or user pregnancy rates were reported. The study included only 126 sponge users. The Yugoslavian study[3] included 403 subjects from two clinics and reported no significant differences between the cumulative 12-month method pregnancy rates of parous and nulliparous sponge users. No data were provided on user pregnancy rates.

If the 13 pregnancies for which there was insufficient information to classify as either method or user failures had been apportioned between method and user failures, lower method and higher user pregnancy rates would have been obtained. Failure to adequately wet the sponge before insertion may be one factor responsible for some of the method failures. When the phase III trials were initiated in 1979, the importance of adequately wetting the sponge to activate the release of spermicide was not fully appreciated. As experience was gained with use of the sponge more emphasis has been placed on adequate wetting before its insertion. This seems to have had a positive effect on the incidence of reported pregnancy.

The continued evaluation of pregnancy rates associated with use of the Today contraceptive sponge shows that it provides women with an adequate level of protection against pregnancy regardless of their age or parity.

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Treatment of nonmetastatic gestational trophoblastic disease with oral methotrexate

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Fifteen patients with nonmetastatic gestational trophoblastic disease were treated solely with methotrexate given orally rather than intramuscularly. Remission, defined as a β-human chorionic gonadotropin titer of <5 mIU/ml for 3 consecutive weeks, was attained in 13 (87%) of the 15 patients. Level of toxicity was acceptable. Patient comfort, convenience, and less time off work and in the physician's office are significant advantages to this efficacious, well-tolerated method of therapy. (AM J OBSTET GYNECOL 1987; 157:1166-8.)

Key words: Gestational trophoblastic disease, oral methotrexate

The treatment of gestational trophoblastic disease has advanced significantly since the initial report of Li et al.[1] describing the efficacy of methotrexate. Today, patients with the nonmetastatic form have remission rates of 100% and low (2.1%) relapse rates.[2] Traditional therapies for nonmetastatic gestational trophoblastic disease include a 5-day course of low-dose intramuscular methotrexate, a 5-day course of daily intravenous dactinomycin (actinomycin D),[3] or an 8-day regimen alternating high-dose intramuscular methotrexate with folinic acid rescue.[4] These regimens require hospitalization or daily visits to the physician's office.

Recent investigations into the treatment of nonmetastatic gestational trophoblastic disease concern regimens that are convenient, cost-effective, easily administered, minimally toxic, and yet maintain the established, excellent cure rate. Although the initial success of Li et al. in treating gestational trophoblastic disease included the use of oral methotrexate,[1] there has been very little subsequent interest in this route of administration. This retrospective report details our experience with only oral methotrexate to treat patients with nonmetastatic gestational trophoblastic disease.

Material and methods

Records of all patients with any form of gestational trophoblastic disease treated at the University of Alabama Medical Center in Birmingham from 1973 to 1984 were reviewed. Fifteen patients with nonmetastatic gestational trophoblastic disease were treated with methotrexate by mouth as their only means of therapy. The standard dose of 0.4 mg/kg with a maximum of 25 mg was given once daily for 5 consecutive days with a 9-day interval before the next course. All patients received one course of therapy after the first β-human chorionic gonadotropin (β-hCG) titer of <5 mIU/ml. Patients medicated themselves with the daily prescribed afternoon dose of methotrexate. They were seen by physicians and hematologic and hepatic laboratory studies were obtained on the first and fourth treatment days as indicated. Most of the patients were treated by their local physicians in consultation with gynecologic oncologists at this institution. No patient was hospitalized for treatment. Doses and treatment intervals were modified according to level of toxicity.

Results

The median age of the patients was 22½ years. Chemotherapy was started in one patient who had a choriocarcinoma in the initial endometrial curettage specimen. The other 14 patients had evacuated molar pregnancies evacuated, and subsequent stabilization or elevation of their β-hCG titers mandated a work-up and chemotherapy. Pretreatment evaluation included chest x-ray films, brain scan, or computed tomography and liver scan or an abdominal computed tomography scan.

Thirteen of the 15 patients (87%) receiving oral methotrexate as their only form of chemotherapy attained biochemical remission. The median number of chemotherapy courses was 2.88 (range two to five). Seven of the 15 patients experienced mucositis, but only

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three needed a dose reduction. No patient experienced granulocytopenia (<3000 white cells/mm³), thrombocytopenia (<100,000 platelets/mm³), or hepatic toxicity (any liver function test greater than two times normal). The two patients with resistant gestational trophoblastic disease were cured with 5-day courses of actinomycin D; each needed and three courses, respectively.

The median β-hCG at the start of oral methotrexate was 2745 mIU/ml (range 48 to 58,000 mIU/ml). There were no relapses, which were defined as recurrent gestational trophoblastic disease within 1 year of the first β-hCG titer of <5 mIU/ml. The median follow-up is 5.3 years (range 1.96 to 11.8 years).

Comment

Treatment of nonmetastatic gestational trophoblastic disease with conventional methods has an excellent cure rate, and yet efforts have been made to decrease toxicity and increase patient convenience and cost-effectiveness. In 1967 Hammond et al.[5] reported a sustained remission rate of 93% with the use of intramuscular methotrexate. In 1975 Osathanondh et al.[6] revealed a 94% remission rate using a 5-day course of intravenous actinomycin D. Unfortunately, almost one half of the patients treated with actinomycin developed alopecia.[7] More recent information by Smith et al.[8] revealed an overall remission rate of 82% in the treatment of nonmetastatic gestational trophoblastic disease with intramuscular methotrexate; 7.7% of patients required a change because of resistance to methotrexate, and 10.3% needed a change because of methotrexate toxicity. Using high-dose intramuscular methotrexate with folinic acid rescue, the same group of investigators achieved a remission rate of only 72.6%, although Berkowitz et al.[4] showed an overall sustained remission rate of 89% using a similar regimen. Toxicity rarely necessitated a change of therapy when using intramuscular methotrexate with folinic acid rescue[4,8]; however, these patients are often hospitalized during the days of treatment.

Alternative routes of therapy include the recent prospective report by Petrilli et al.[9] from the Gynecologic Oncology Group that showed a 94% remission rate with the use of biweekly outpatient pulse actinomycin D in patients with nonmetastatic gestational trophoblastic disease. However, in an earlier report, Schlaerth et al.[10] reported only a 75% remission rate with similar regimen of pulse actinomycin D therapy. Nausea and vomiting were the chief toxicities but were felt to be acceptable in frequency and severity. Wong et al.[11] reported a 100% remission rate with oral VP16 for gestational trophoblastic disease, but all patients had nausea and 92% had total alopecia.

Our remission rate of 87% with only oral methotrexate is comparable with those obtained with intramuscular methotrexate, intramuscular methotrexate with folinic acid rescue, and pulse actinomycin D. The toxicities associated with oral methotrexate are mild, and the overall recurrence rate of 0% compares favorably with the 6.3% and 1.2% rates for intramuscular methotrexate and methotrexate with folinic acid rescue, respectively.[4,8]

Oral methotrexate use in malignancies other than gestational trophoblastic disease has been studied, but different doses and periods of treatment are used. Henderson et al.[12] demonstrated that low doses of methotrexate are completely absorbed from the gastrointestinal tract, whereas higher doses have a more prolonged and incomplete absorption phase. Significant interindividual variability in methotrexate levels after oral administration has been demonstrated,[13] but this was in the prolonged 1- or 2-year maintenance phase of leukemia treatment. Mucositis may interfere with the absorption of oral methotrexate, although this did not appear to have clinical significance in our patients.

We recommend that methotrexate be given as a single dose in the late afternoon. Subdivided doses should not be given, since they cause higher prolonged serum levels and could result in more toxicity.[14] In addition, methotrexate should be given 1 hour before or 2 hours after meals to ensure absorption. Finally, methotrexate should not be administered with salicylates or sulfa drugs, since these may displace the methotrexate from plasma proteins, resulting in higher serum concentrations.[15]

Oral methotrexate has the significant advantages of patient convenience and comfort, decreased cost, and less interference with the patient's daily work schedule. Compared with other regimens, the remission and recurrence rates are excellent and the drug is very well tolerated. Patients who do not improve with oral methotrexate are readily cured with actinomycin D and are not in jeopardy of having resistant disease. A future report will describe our experience treating patients with nonmetastatic gestational trophoblastic disease with one of the more traditional routes followed by oral methotrexate cycles. Controlled prospective studies are needed to provide further information regarding this convenient and simple route of therapy.

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Sonographic diagnosis of a bladder polyp

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Presented is a case of a bladder polyp diagnosed by ultrasound and removed by transurethral resection.
(Am J Obstet Gynecol 1987;157:1168-9.)

Key words: Bladder, ultrasound, polyp

Inflammatory polyps of the bladder are not uncommon, but are usually diagnosed at the time of cystoscopy for recurrent urinary complaints.

Case report

A 44-year-old woman, gravida 4, para 2-0-0-2, presented for follow-up 3 months after a uterine curettage done outside the United States and performed because of a blighted ovum. Physical examination results were within normal limits, but on inspection of the original ultrasound pictures a polyplike structure was noted in the bladder (Fig. 1). The patient was totally asymptomatic but gave a history of previous bladder infections. She was referred to a urologist, who confirmed the finding. A transurethral resection of the polyp was performed. The polyp measured 1.3 by 0.7 by 0.5 cm and on histologic examination was diagnosed as severe glandular cystitis.

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Comment

In long-standing chronic inflammation, small areas of bladder mucosa may become buried, causing small cystic inclusions. Occasionally the epithelium undergoes metaplasia and begins to become columnar mucus secreting, giving rise to glandular cystitis.[1]

The initial cystoscopic diagnosis of a malignancy was incorrect in this case. Had it been correct and if the malignancy was missed, the final result might have been very different.

Sonography has been used increasingly as a noninvasive preliminary diagnostic tool to investigate causes of hematuria, dysuria, and voiding difficulties in children.[2] In adults, however, radiology and cystoscopy have remained the diagnostic modalities of choice. That the sonographic diagnosis of an inflammatory bladder polyp is rare is supported by the absence of reports in the literature. Benecerraf et al.[3] reported on four bladder tumors found incidentally in 3000 pelvic ultrasound studies. However, all of these were malignant tumors.

In routine gynecologic sonography, a full bladder is used to enable one to view the pelvic organs more clearly. It may therefore behoove the examiner, as illustrated by this case, to use this opportunity to examine

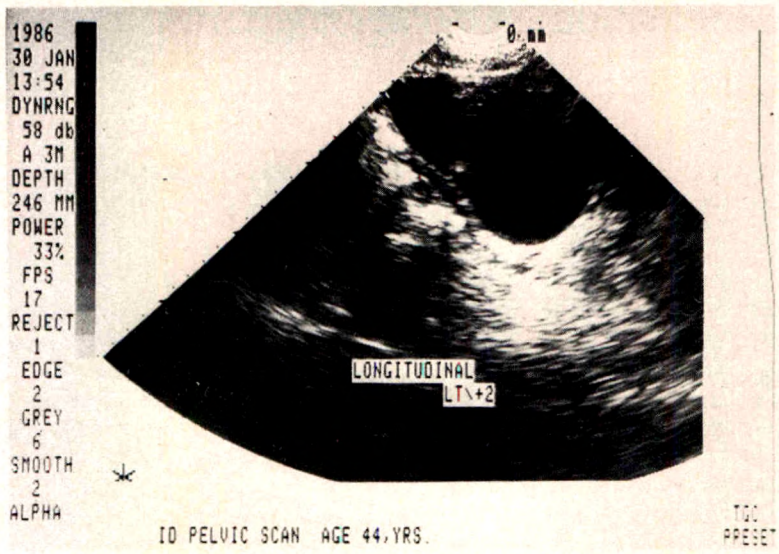


Fig. 1. Longitudinal view of bladder showing polyp posteriorly.

the bladder systematically before proceeding to the gynecologic examination, not merely to use the bladder as a "window." Conversely, however, it should be stressed that ultrasound should not be used as a substitute for cystoscopy in the diagnosis of bladder tumors, as proved tumors have been missed by this modality.[4]

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Sonographic evaluation of the cervix during ovulation induction

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With the induction of ovulation, clomiphene citrate and human menopausal gonadotropin have the potential to stimulate the endocervical glands. We have found that the degree of mucus production can occasionally be quite large and hence is detectable by sonographic evaluation as an anechoic cervical mass. The incidence of this specific ultrasonic finding was found to be approximately 5%. This physiologic process should be differentiated from disease states affecting the cervix. (Am J Obstet Gynecol 1987;157:1170-4.)

Key words: Ultrasound, cervix, ovulation induction

Historically, ovulation has been monitored by clinical parameters. With the development of radioimmunoassays, the hormonal assessment of ovulation became possible. During the 1970s, the development of high-resolution ultrasonography permitted the endocrinologist to: (1) observe the ovary during the menstrual cycle, (2) identify the dominant follicle, and (3) determine the probable time of ovulation.[1-3] In vitro fertilization programs have come to rely heavily on ultrasound examination to not only provide a reliable measure of follicular growth but also to direct transabdominal or transvaginal egg retrieval.[4, 5]

Ovulation-inducing agents (clomiphene citrate and human menopausal gonadotropin) also play a key role in in vitro fertilization programs by increasing the number of preovulatory follicles available for retrieval. These drugs may indirectly effect the cervix by altering the concentration of estrogen and progestogen.

We present our findings relative to the sonographic evaluation of the cervix during the induction of ovulation with either clomiphene citrate or human menopausal gonadotropin.

Material and methods

One hundred two consecutive patients receiving clomiphene citrate and 344 patients receiving human menopausal gonadotropin for ovulation induction were studied.

Ultrasound examinations were performed with real-time equipment with a 3.5 MHz transducer. A moderately full bladder was required as the acoustic window through which normal pelvic anatomy could be viewed.

Results

Table I outlines the number of patients taking either clomiphene citrate or human menopausal gonadotropin who had sonographically detectable cervical mucus during an induced cycle. Clomiphene citrate and human menopausal gonadotropin appear to have a comparable effect on endocervical gland production: 4.9% of patients in each group had sonographically detectable cervical mucus.

The degree of bladder filling plays a critical role in the prevalence rate obtained. If the bladder is overly distended, the cervix is compressed and cervical mucus is not apparent (Fig. 1, *A*). After partial voiding, the same patient may be found to have a significant amount of endocervical mucus (Fig. 1, *B*).

The amount of cervical mucus present on sonographic evaluation may vary from several small adjacent anechoic areas to a single large mass (Figs. 2, and 3, *A* and *B*).

Table II outlines the history, diagnosis, therapy, and estradiol levels of three study patients on human menopausal gonadotropin. Sonographically, the degree of cervical mucus production was unrelated to the levels of estradiol.

Comment

Histologically, the cervix is composed of a complicated system of longitudinal, oblique, and transverse mucosal folds that extend into the subadjacent connective tissue.

In the early follicular phase of a normal cycle, the endocervical glands are short and have a relatively simple pattern. However, in the late follicular phase, the

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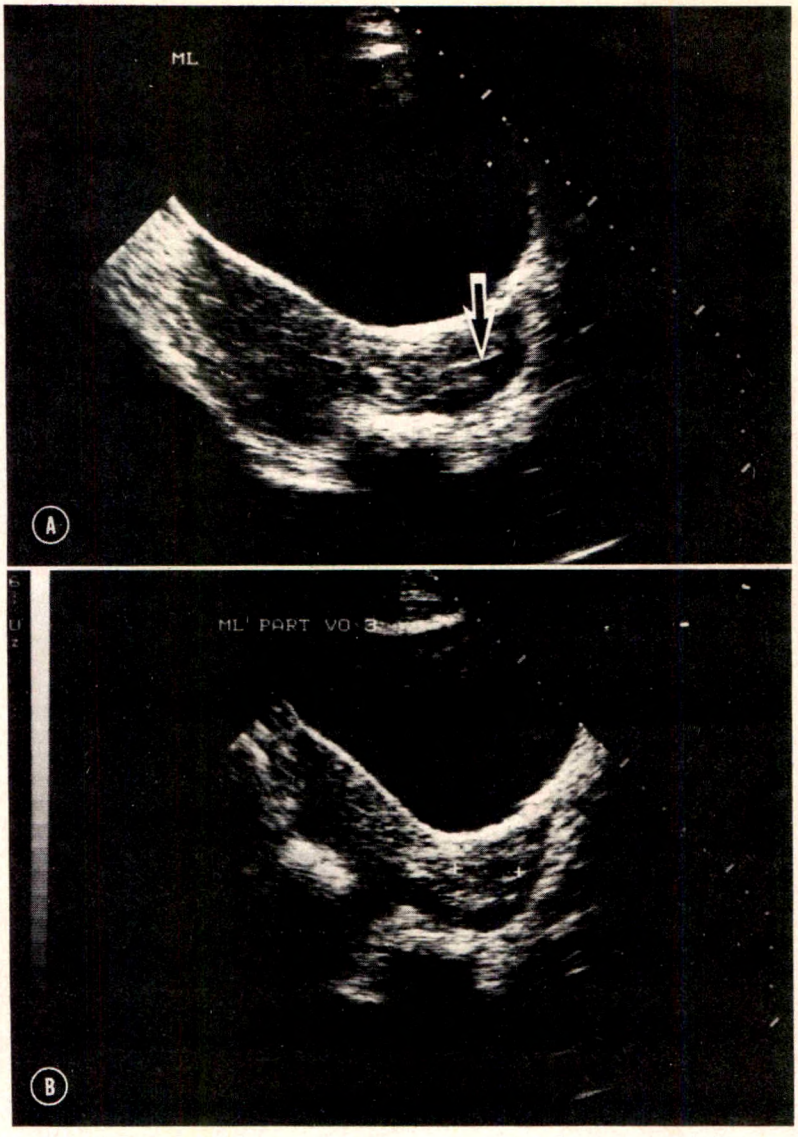


Fig. 1. A longitudinal scan of the uterus in a patient taking human menopausal gonadotropin. **A,** With a full bladder (*arrow* points to the endocervical canal). **B,** After partial voiding; the gradicules are on the now apparent endocervical mucus.

glands become tortuous; they invaginate further into the cervical connective tissue, the columnar epithelium achieves its maximal height, and mucus production is at its peak. Vascularization of the cervix is improved, and the texture of the cervical connective tissue becomes looser. Anatomically, the external cervical os widens during the menstrual cycle and achieves its maximal width immediately before ovulation.

The appearance of cervical mucus has been scored by Insler et al.[6] This system takes into consideration the quantitative and qualitative changes that occur in cervical mucus during different phases of the menstrual cycle. Thus the quantity of mucus, the presence or absence of ferning, spinnbarkeit, and the appearance of the cervix are assessed.

Table I. Prevalence of cervical mucus after ovulation induction

	No. of patients	*Cervical mucus*	
		No.	*%*
Clomiphene citrate	102	5	4.9
Human menopausal gonadotropin	344	17	4.9

Clomiphene citrate is a nonsteroidal estrogen antagonist. Its administration results in increased secretion of luteinizing hormone and follicle-stimulating hormone. As a result, follicles that would ordinarily

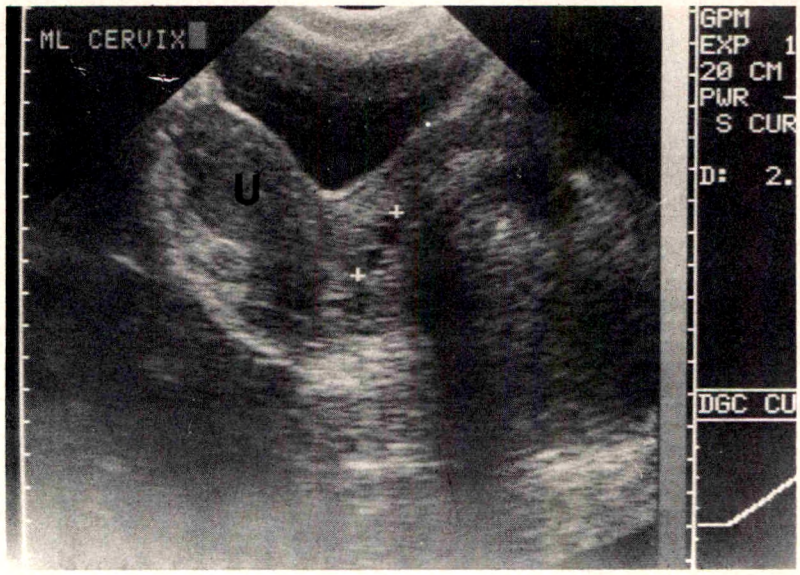


Fig. 2. A longitudinal scan of the uterus *(u)* in a patient taking human menopausal gonadotropin. *Markers* indicate the bilobed echo-spared area of mucus accumulation within the cervix.

Table II. Clinical description of three patients undergoing ovulation induction for the treatment of infertility who were studied by ultrasonographic examination

Age (yr)	*Gravida*	*Para*	*Duration of infertility (yr)*	*Diagnosis*	*Treatment*	*Peak estradiol (pg/ml)*
35	4	0	7	LPD	hMG	314
31	1	0	3	Anovulation	hMG	1730
33	0	0	3½	Anovulation	hMG	511

LPD = Luteal phase defect; hMG = human menopausal gonadotropin; CC = clomiphene citrate.

become atretic after the selection of a dominant follicle continue to grow and mature. Although ovulation rates are reported to be between 50% and 80% while taking clomiphene citrate, significantly lower rates of conception are achieved.[7] Thick tenacious mucus caused by the antiestrogenic effect of clomiphene has been proposed by several authors as the etiologic mechanism for this finding.[8] However, Hancock and Oakley[9] found that this effect disappeared within 1 week after the medication was discontinued. Gysler et al.[10] discovered additional infertility factors in 159 of 182 patients (87.4%) who ovulated but did not conceive while taking clomiphene citrate. The authors also stated that better cervical mucus was present in the patients receiving clomiphene citrate than in the general population of infertility patients referred to their clinic. O'Herlihy et al.[11] have also suggested that the antiestrogenic effects of clomiphene have relatively little effect on the cervical mucus score.

Stimulation of the cervical glands with human menopausal gonadotropin is caused by the estrogen produced by the maturing follicles. Since a greater number of follicles are usually stimulated with human menopausal gonadotropin than with clomiphene, the levels of estrogen achieved during human menopausal gonadotropin–induced cycles are frequently higher. Therefore one would anticipate a greater stimulation of the cervical glands with human menopausal gonadotropin than with clomiphene citrate.

The reported effects of ovulation-inducing medications on cervical gland production have failed to date to take into consideration the target organ. Sanborn et al.[12] have found specific estrogen-binding proteins in the human cervix. Clomiphene citrate may, for example, act locally to block receptors in the endocervical epithelium. The quantity of receptors (and blockers), as well as their binding properties, may vary from patient to patient and cycle to cycle, thereby producing the variable effects noted by different groups. Our findings add credence to this assumption. A noticeable amount of mucus was not apparent on sonographic examination until after 5 days of clomiphene or human

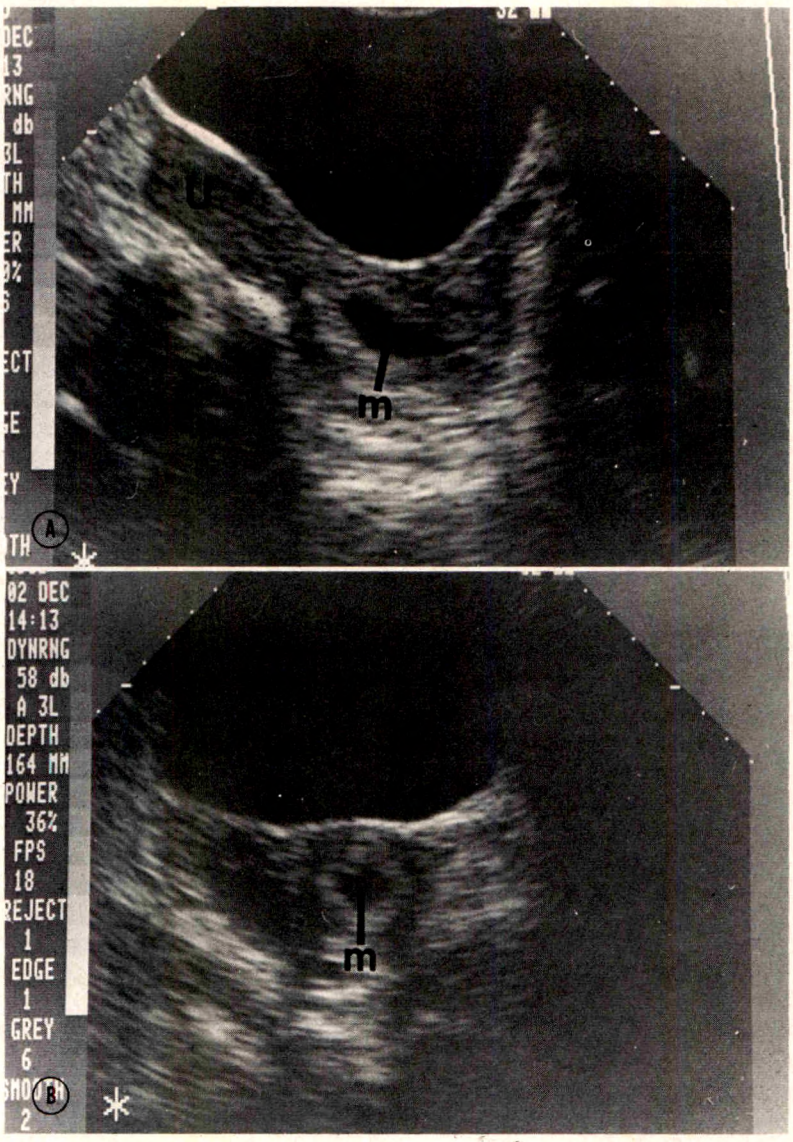


Fig. 3. Sonographic evaluation of the cervix after human menopausal gonadotropin. **A,** Longitudinal scan. Mucus accumulation in the cervix is readily apparent. (u = Uterus; m = mucus.) **B,** Transverse scan of the cervix. (m = Mucus.)

menopausal gonadotropin. Although this suggests an association with increased estrogen production, the presence or absence of ultrasonically detectable cervical mucus was not directly related to a specific estrogen level obtained in a given cycle (Table I). Consequently, a patient may have several induced cycles without any noticeable effect on the cervix followed by a cycle in which the retention of cervical mucus is particularly prominent. We have found that the retention of cervical mucus may last from one to 10 cycles and in some instances can be quite large (Fig. 3, *A* and *B*).

The practice of ultrasonographic monitoring during ovulation induction has increased. As a result, the sonographer should be aware of not only the follicular changes associated with stimulated cycles but also with the possibility of the cervical changes previously noted. The recognition of the iatrogenic cause of the latter findings will alleviate not only the patient and physician's anxiety concerning the presence of a cervical mass, but it will also prevent further unnecessary testing.

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A prospective randomized study of the aggressive management of early labor

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The aggressive management of early labor has been suggested as a means to lower the cesarean section rate for dystocia. The aim of our study was to prospectively evaluate a protocol of early intervention in primigravid women with infrequent uterine contractions during early labor in relation to the course of labor, the cesarean section rate, and the perinatal morbidity. In our population the active management of labor did not alter the mode of delivery or the perinatal outcome. Furthermore, the course and duration of labor were not significantly different between the management and control groups. (Am J Obstet Gynecol 1987;157:1174-7.)

Key words: Labor, active management; dystocia; cesarean section

The rate of cesarean birth has exceeded 20% nationally. The largest increase has been in the group characterized by the diagnosis of dystocia. Approximately 50% of all primary cesarean sections are performed because of dystocia while the remainder are done because of malpresentation, fetal distress, or medical indications.[1] This dramatic rise in operative delivery rate in the United States has not been paralleled in other countries.[2-4] The experience in Ireland has been a stable incidence rate with the incidence of dystocia being one tenth that of the United States.[5, 6] This difference has been attributed to the philosophy of active management of labor.[5, 7, 8] The purpose of this study was to assess an aggressive approach to early labor in a prospective randomized fashion to determine if such treatment could alter the cesarean birth rate in an American hospital serving a primarily indigent population.

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Material and methods

One hundred fifty primigravid patients who presented to the obstetric suite at Tampa General Hospital between September 1, 1985, and June 20, 1986, were entered into the study protocol. Eligible patients were treated according to the study protocol as approved by the institutional review boards of both the hospital and the University of South Florida College of Medicine. All subjects were low-risk primigravid women at 37 to 42 weeks' gestation who met our standard criteria for admission to the labor suite (uterine contractions ac-

Table I. Population characteristics

	Early intervention group	*Control group*	p *Value*
Race (%)*			
White	54.7	66.7	
Black	37.4	21.3	
Hispanic	8.0	10.7	
Age (yr)	19.5 ± 0.4	20.6 ± 0.4	NS†
Dilation (cm)	2.6 ± 0.1	3.0 ± 0.1	0.03†
Effacement (%)	82.3 ± 2.3	85.3 ± 2.5	NS†
Station	−2.2 ± 0.08*	−2.2 ± 0.08	NS†

*NS, χ^2.
†Student's paired *t* test.

Table II. Mode of delivery

	Early intervention group (N = 75)*		*Control group* (N = 75)*	
Mode of delivery	*n*	%	*n*	%
Vaginal	65	87	64	85
Spontaneous	59	79	61	81
Low forceps	6	8	3	4
Cesarean section	10	13	11	15
Arrest—stage 1	4	5	4	5
Arrest—stage 2	4	5	5	7
Fetal distress	2	3	2	3

*NS, χ^2.

companied by cervical dilation of 3 cm or ruptured membranes). Additionally, all subjects demonstrated an inadequate pattern defined as a frequency of less than three contractions lasting 40 seconds each in a 10-minute time period.

Eligible patients were required to render informed consent before randomization, which was accomplished by alternating subjects to aggressive management or control groups. Seventy-five patients were assigned to each group in this fashion.

The early aggressive management protocol consisted of amniotomy if required, insertion of a fetal electrode and an intrauterine pressure cannula, and oxytocin infusion. This was accomplished within 30 minutes of the patient's presentation to the labor suite.

Patients in the control group underwent external fetal monitoring. Internal electronic monitoring was reserved for those with nonreassuring fetal heart rate patterns or those patients requiring augmentation. Augmentation was begun in the control group when cervicographic analysis revealed secondary arrest of dilation (no increase in cervical dilation) of >2 hours' duration in the active phase or arrest of descent (no change in station) of 1 hour's duration in the second stage of labor in the presence of an inadequate contraction pattern.

Oxytocin infusion followed a standardized protocol consisting of an initial infusion of 1 mU/min with an increase of 1 mU/min every 30 minutes until an adequate contraction pattern was achieved.

In both groups cesarean delivery for the diagnosis of cephalopelvic dysproportion was performed in the case of arrest of dilation of 3 hours' duration or arrest of descent for 2 hours' duration in the presence of an adequate contraction pattern.

Cesarean delivery because of fetal distress was performed in cases of fetal acidemia or bradycardia that were unresponsive to positional change and cessation of oxytocin infusion if necessary.

Midforceps deliveries were not performed in enrolled patients but low-forceps deliveries were allowed at the physician's discretion.

For cervicographic analysis of labor patterns, the latent phase was defined as the time from presentation until the start of the active phase (the time period during which the rate of cervical dilation was maximal). The deceleration phase was the time period that elapsed between the end of the active phase and complete dilation.

Entries for maternal characteristics, labor progression, and delivery were recorded on data sheets and analyzed by means of the Statistical Analysis Systems statistical analysis package. Differences between groups were assessed by Student's unpaired *t* test and χ^2 analysis with a $p < 0.05$ level considered as significant.

Results

The study and control groups were similar in composition with no significant differences in race or age (Table I). The initial cervical dilation of the study group (2.6 cm) was slightly less than that of the control women (3.0 cm) but there was no difference in either effacement or station.

The groups demonstrated remarkable similarity in the mode of delivery (Table II). The overall rate of cesarean birth was 14%. Eighty-seven percent in the early intervention group (65/75) versus 85% (64/75) of the control group were delivered vaginally. In those patients delivered vaginally, 8% in the early intervention group versus 4% in the control group required forceps. Twenty-eight of 75 patients in the control group ultimately received oxytocin augmentation. Cesarean deliveries were performed because of dystocia in eight patients (10.7%) in the early intervention groups and in nine patients (12.0%) in the control group. The distribution of cesarean sections for arrest of labor in the first or second stage of labor was also similar in both groups (Table II).

Table III. Characteristics of labor*

	Early intervention group (N = 66)	*Control group (N = 65)*	p *Value†*
Duration of labor (hr)			
Latent period	1.42 ± 0.33	2.15 ± 0.29	NS
Active phase	4.38 ± 0.36	4.14 ± 0.36	NS
Deceleration phase	0.27 ± 0.07	0.27 ± 0.06	NS
Dilation rate (cm/hr)			
Active phase	1.94 ± 0.15	2.05 ± 0.16	NS
First stage	6.10 ± 0.39	6.60 ± 0.36	NS
Second stage	1.04 ± 0.12	1.01 ± 0.11	NS

Values are mean ± SEM.
*Subjects achieving vaginal delivery.
†Student's unpaired *t* test.

Table IV. Outcome

	Early intervention group	*Control group*	p *Value**
Birth weight (gm)	3242 ± 57	3224 ± 57	NS
1 min Apgar score	8.33 ± 0.12	7.98 ± 0.15	NS
5 min Apgar score	8.97 ± 0.06	8.95 ± 0.06	NS
Cord pH	7.31 ± 0.01	7.31 ± 0.01	NS

*Student's unpaired *t* test.

The total duration of labor of all subjects in both groups did not differ significantly (7.8 ± 3.7 hours in the study group versus 8.0 ± 3.3 hours in the control group). As expected, subjects delivered by cesarean section because of arrest disorders had considerably longer labors (12.4 ± 4.2 hours) than those delivered vaginally (7.4 ± 3.1 hours) but the duration of labor in these subgroups did not differ between the study and control groups.

Detailed analysis of the cervicographic labor pattern was restricted to those patients achieving vaginal delivery (Table III). The duration of the first and second stages of labor in the two groups did not exhibit a statistical difference. Further analysis showed that the course of labor, when plotted on the standard Friedman labor curve, was comparable. The slope of dilation versus time showed no statistical difference in the latent phase, active phase, and deceleration phase. The number of patients undergoing cesarean deliveries because of dystocia in both groups was small but the characteristics of labor were similar.

Table IV illustrates the comparison of groups by birth weight, Apgar scores, and cord pH. The birth weights were similar as were 1-minute and 5-minute Apgar scores and the values for cord pH. When infants of >4000 gm were isolated, it was found that two of the four in the early intervention group versus both of those in the control group were delivered vaginally.

Comment

The perinatal mortality rate in the United States has fallen markedly over the past 20 years. This drop has coincided with an increasing rate of cesarean birth. A possible cause-and-effect relationship between these trends has been actively debated.[12, 13]

The active management of labor was originally proposed as a means to prevent prolonged labor.[7-10] As the divergence in the rate of operative intervention because of cephalopelvic disproportion between the United States and the British Isles became apparent, some authors attributed this difference to the disparate methods of labor management. O'Driscoll et al.[5, 8, 9] have published a number of reports associating early oxytocin augmentation with a low incidence of cesarean birth for dystocia while maintaining a low perinatal mortality rate.

A recent report comparing the experience at the National Maternity Hospital, Dublin, and the Parkland Memorial Hospital, Dallas, however, cites differences in racial composition and parity as a likely source of the discrepancy in cesarean delivery rate.[6] Nonetheless, preliminary reports have attributed a fall in the rate of cesarean birth as a result of dystocia to the implementation of active management in an American hospital.[14, 15]

In this study we sought to control for differences in the patient population and other, less clearly defined variables by using a prospective, randomized design limited to the subset of most interest, the nulliparous patient in early labor at term with infrequent uterine contractions. Management between groups was identical except for the early implementation of oxytocin infusion and amniotomy.

The study was not designed to replicate the active management protocol outlined by O'Driscoll. Instead, this protocol of early treatment of inadequate uterine

contractions was instituted to determine whether early aggressive treatment would shorten labor and decrease the cesarean section rate when compared with expectant management for variable periods of time. Presently, at our institution as well as many others, patients are treated expectantly for variable periods of time with active intervention being undertaken under different circumstances. We attempted to control for these variables by prospectively randomizing these patients and treating them with strict adherence to the study protocol.

In this environment there was a remarkably similar performance between the two groups. Not only was the incidence of cesarean birth nearly identical, but no significant differences were noted in the length of labor, duration of the phases of labor, or the rate of cervical dilation. These similarities in the progression of labor are important to note as they are likely to be more sensitive to major changes in efficacy then the rate of cesarean birth for dystocia. In a study of this design, assurance of a lack of reduction in the rate of cesarean birth from 12% to 8% (33% reduction) at a probability level (power) of 0.2 would require 900 subjects in each group. The difficulties in a study of this magnitude are obvious. Because of the greater precision in the duration and progression of labor, however, we can exclude, at the same level of certainty, reductions of >33% in the duration of the active phase or >30% in the rate of dilation.

In summary, we have conducted a randomized, prospective trial of early intervention in primigravid women in a large teaching service. In our population early intervention failed to reduce the incidence of cesarean birth as a result of dystocia or to affect the duration of labor. We recommend careful consideration before the widespread use of such methods.

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Breast stimulation test and oxytocin challenge test in fetal surveillance: A prospective randomized study

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Fifty healthy gravidas with uncomplicated singleton pregnancies at a gestational age of 35 to 42 weeks were assigned at random for the performance of either a breast stimulation test or an oxytocin challenge test after a nonreactive nonstress test. A satisfactory contraction stress test was achieved in 21 of 25 women in the breast stimulation test group and in 24 of 25 women in the oxytocin challenge test group (nonsignificant difference). Significant differences were found in the cumulative rate of achieved contraction stress tests: in the breast stimulation test group 17 of 21 after 20 minutes and 21 of 21 at 50 minutes whereas in the oxytocin challenge test group only four of 24 at 20 minutes, eight of 24 at 50 minutes, and 21 of 24 at 150 minutes. The mean duration for the achievement of a contraction stress test was 20.9 ± 11.5 minutes for the breast stimulation test group and 81.3 ± 48.4 minutes in the oxytocin challenge test group. Uterine hyperstimulation occurred once in each group. No gravida went into labor within 24 hours. Breast stimulation test is a satisfactory alternative to the oxytocin challenge test, is less time-consuming, and is simpler to perform. (Am J Obstet Gynecol 1987;157:1178-81.)

Key words: Fetal monitoring, oxytocin challenge test, breast stimulation test

The contraction stress test and the nonstress test are well-established diagnostic tools for the prenatal evaluation of the fetoplacental unit.[1-3] For practical reasons the nonstress test has been used in most centers as the first diagnostic test followed by a contraction stress test if a nonreactive pattern was obtained.[4, 5] A contraction stress test is usually performed with either spontaneous uterine contractions or after induction of uterine activity by exogenous oxytocin infusion (oxytocin challenge test).[6] Recently several studies have indicated the efficacy of nipple stimulation for the initiation of uterine contractions, suggesting that the breast stimulation test is a convenient method of performing a contraction stress test.[7-10] The breast stimulation test, however, has been associated with a high incidence of uterine hyperstimulation.[11, 12]

The purpose of this study was to compare prospectively the use of intravenously administered oxytocin to nipple stimulation for the induction of uterine activity in the performance of a contraction stress test.

Material and methods

During the study period nonstress tests were performed on every gravida at a gestational age of 35 to 42 weeks either as a routine screening procedure at term or after the perception of decreased fetal movements. Subjects with multiple pregnancies, diabetes, hypertension, intrauterine growth retardation, or a pathologic nonstress test (defined as any type of deceleration) were excluded from this study. Of the 210 subjects fulfilling these criteria, a nonreactive nonstress test was present in 50 patients. A reactive pattern was defined as two fetal heart rate accelerations in any 20-minute period, with each acceleration rising at least 15 bpm above baseline and lasting for at least 15 seconds. The 50 patients with a nonreactive nonstress test underwent contraction stress tests. The participants were randomly divided; 25 underwent an oxytocin challenge test and the other 25 women had a breast stimulation test.

All testing was performed with the patient in the left lateral recumbent position. The procedures included an initial 15-minute recording to determine fetal baseline heart rate and variability. Oxytocin was administered via an infusion pump at a rate of 2 mIU/min. The dosage was raised by 1 mIU/min every 10 minutes until three uterine contractions of at least 40-second duration were recorded during a 10-minute period. At this stage oxytocin administration was discontinued, and fetal heart rate was monitored for an additional 20 minutes.

After the initial 15-minute recording, the women allocated to the nipple stimulation group were instructed to rub the nipple of one breast for 15 minutes. If no adequate uterine activity was recorded, then both nip-

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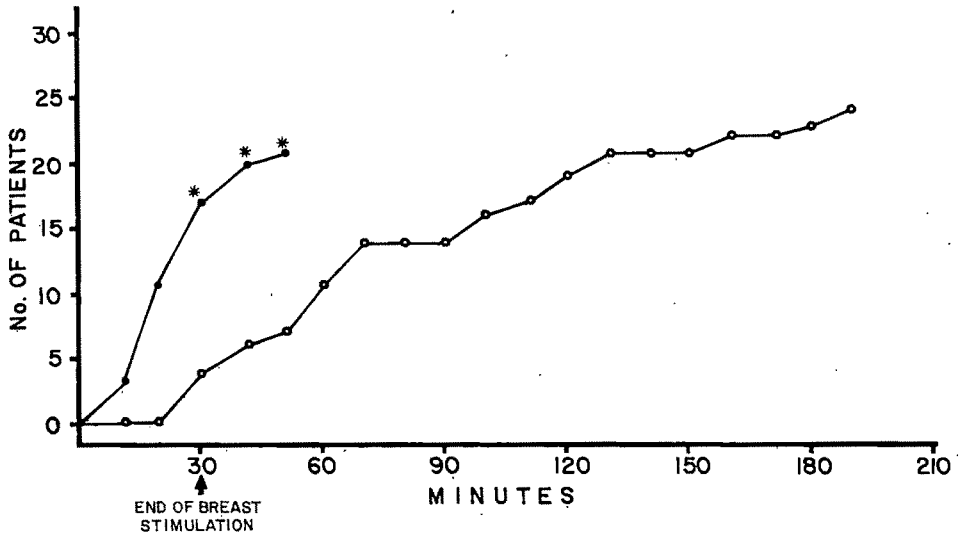


Fig. 1. The cumulative success rates of the breast stimulation test (*closed circles*) and the oxytocin challenge test (*open circles*). (*Asterisk* = $p < 0.01$).

ples were stimulated for another 15 minutes. The fetal heart rate was monitored for 20 minutes after cessation of breast stimulation.

Both the oxytocin challenge test and breast stimulation test were characterized as negative, positive, suspicious (irregular late or variable decelerations), hyperstimulated, and failed. Informed consent was obtained from all women enrolled in the study. All participants were instructed by a nurse before the start of the procedure.

Statistical analysis. Statistical evaluation of the data was accomplished by the unpaired *t* test, the χ^2 test (Yates correction), and the Fisher exact test where applicable. Differences were considered statistically significant with $p < 0.05$.

Results

The mean age of the two study groups was similar (28 ± 5.2 years for the oxytocin challenge test group and 29.7 ± 6.5 years for the breast stimulation test group). The parity of the two groups was similar. The distribution of the participants according to gestational age at the time of the study is shown in Table I. The success rates of the tests in the two groups according to gestational age are depicted in Table II. A satisfactory contraction stress test (three contractions per 10 minutes) was achieved in 21 (84%) gravidas in the breast stimulation test group and in 24 (96%) women in the oxytocin challenge test group. This difference was not statistically significant. All women in both groups acted in accordance with the study protocol, and none of the failures to achieve a contraction stress test could be attributed to inadequate compliance of the participants.

Hyperstimulation of the uterus occurred once in each group. In both subjects at the end of the uterine tetany, the contraction stress test was accomplished without adverse results.

Table I. Distribution of the participants according to gestational age

Gestational age (wk)	*BST group*	*OCT group*
≤37	5	6
38-40	11	14
≥41	9	5
Total	25	25

BST = Breast stimulation test; OCT = oxytocin challenge test.

The time intervals from the initiation of the test until adequate uterine stimulation are depicted in Fig. 1. In 10 (40%) gravidas of the breast stimulation test group, an adequate contraction stress test was obtained during the initial 15-minute period (single-nipple stimulation). Since according to the study protocol the breast stimulation test was planned for a duration of 30 minutes, we compared the number of satisfactory tests achieved during the first 30 minutes with each method. In the oxytocin challenge test group only four women had completed a contraction stress test as desired during this time period compared with 17 subjects in the breast stimulation test group ($p < 0.01$). Four additional patients in the breast stimulation test group achieved a contraction stress test during the 20-minute period after the cessation of nipple stimulation. At that time (50 minutes) only seven subjects in the oxytocin challenge test group had achieved a contraction stress test ($p < 0.01$). The mean time interval from the start of the contraction stress test until the achievement of three contractions within 10 minutes was 20.9 ± 11.5 min-

Table II. Outcome in each group in relation to gestational age

	≤37 wk	38-40 wk	≥41 wk	Total (%)
BST				
CST and one nipple stimulated	2	6	5	13 (52)
CST and two nipples stimulated	3	2	2	7 (28)
Tetanic contractions	—	1	—	1 (4)
No. adequate uterine contractions	—	2	2	4 (16)
Total				25 (100)
OCT				
CST achieved	6	12	5	23 (92)
Tetanic contractions	—	1	—	1 (4)
No. adequate uterine contractions	—	1	—	1 (4)
Total				25 (100)

BST = Breast stimulation test; CST = contraction stress test; OCT = oxytocin challenge test.

Table III. Clinical outcome of the contraction stress tests in relation to gestational age*

	≤37 wk		38-40 wk		≥41 wk		Total	
	BST	OCT	BST	OCT	BST	OCT	BST (%)	OCT (%)
Negative CST	4	6	8	11	7	5	19 (76)	22 (88)
Positive CST	—	—	—	1	—	—	—	1 (4)
Suspicious CST	1	—	1	1	—	—	2 (8)	1 (4)
CST not achieved	—	—	2	1	2	—	4 (16)	1 (4)

BST = Breast stimulation test; OCT = oxytocin challenge test; CST = contraction stress test.
*Differences between groups not statistically significant.

utes in the breast stimulation test group and 81.3 ± 48.4 minutes in the oxytocin challenge test group.

The clinical outcome of the tests was similar in the two groups (Table III). Uterine activity subsided shortly after the achievement of the contraction stress test, and no gravida went into spontaneous labor within 24 hours after the tests.

Comment

Stimulation of the mammary nipple may lead to uterine contractions during pregnancy, labor, and the postpartum period presumably via activation of the hypothalamus and the oxytocic neurons that end at the posterior pituitary gland.[13] However, studies have failed to detect significant elevations of endogenous oxytocin levels after nipple stimulation.[14] The contraction stress test has been widely accepted as a diagnostic tool for fetal well-being. During a contraction, uterine blood flow is temporarily decreased, which leads to a decline in fetal oxygenation.[8] Although a healthy fetus tolerates these changes, a fetus already compromised by impaired uteroplacental perfusion may not be able to withstand a further decrease in oxygen supply, which will result in pathologic heart rate changes. The contraction stress test has been performed mainly by the intravenous infusion of oxytocin. The exogenous administration of oxytocin requires an intravascular line, accurate monitoring of the administration rate of the potentially hazardous drug, a hospital bed, and a relatively long lag time from the start of administration until adequate uterine stimulation is achieved. The presented results confirm previous studies that concluded that the breast stimulation test overcomes some of these problems.[7,8] The performance of the breast stimulation test was simple and less time-consuming when compared with the oxytocin challenge test whereas the clinical outcome was similar with both methods.

The safety of the breast stimulation test is controversial. Since the endogenous secretion of oxytocin after nipple stimulation leads to persistent uterine contraction during the postpartum period, similar uterine stimulation prenatally may cause uterine hyperstimulation, tetanic contractions, and fetal compromise.[9] We elected to stimulate the nipple manually. Only one nipple was massaged for the first 15 minutes, and the mammae were stimulated subsequently for another 15 minutes or until the occurrence of three uterine contractions within 10 minutes.

In the present study only one patient in each group (4%) had tetanic contractions during the contraction stress test. Similar favorable results with the breast stimulation test have been reported.[7,9,11] However, Hill et al.[12] have documented a high frequency of exaggerated uterine activity.

The high success rate in achieving an adequate contraction stress test in both groups (84% for the breast

stimulation test and 96% for the oxytocin challenge test) is in accordance with previous studies.[7, 8, 12, 15] However, it should be noted that only seven of the women in the oxytocin challenge test group (28%) had an adequate contraction stress test within the first 50 minutes while all successful breast stimulation tests (21 of 25 patients, 84%) were achieved during the same time period ($p < 0.01$). Four of the contraction stress tests in the breast stimulation test group were achieved within 20 minutes of the cessation of nipple stimulation. Previous reports have emphasized the short time needed for the contraction stress test after the breast stimulation test.[7-9, 15]

This randomized study demonstrates that the breast stimulation test is a satisfactory alternative to the oxytocin challenge test. The breast stimulation test was found to achieve clinical results similar to the oxytocin challenge test, was less time-consuming, and was simpler to perform.

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Intrapartum use of fetal scalp hematocrit in the diagnosis of profound fetal anemia caused by fetomaternal hemorrhage

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A case of massive fetomaternal hemorrhage that caused profound fetal anemia is presented. Timely intrapartum assessment of fetal hematocrit of 15% by means of scalp sampling aided in the delivery of a newborn infant with hemoglobin of 3 gm/dl and hematocrit of 10%. (AM J OBSTET GYNECOL 1987;157: 1182-3.)

Key words: Fetomaternal hemorrhage, fetal anemia, scalp hematocrit

Profound fetal anemia is a life-threatening complication and is usually a result of hemorrhage, hemolysis, or isoimmunization. Antepartum and especially intrapartum diagnosis in suspicious cases may be crucial since the fetuses are not able to tolerate the stress of labor. In this case report, timely intrapartum diagnosis of unexpected profound anemia caused by massive fetomaternal hemorrhage helped deliver a healthy newborn infant.

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Case report

A 24-year-old, gravida 1, blood type A, Rh-positive patient was referred at 39 weeks' gestation because of decreased fetal movements. Pregnancy was uneventful, and ultrasound examination performed 12 hours earlier was normal except for decreased body movements and absent breathing movements. On admission uterine size was consistent with term, and the cervix was soft, effaced 50%, admitting two fingers, and the head engaged. An oxytocin challenge test after a nonreactive nonstress test was positive (Fig. 1, *A*) and an induction of labor was begun. Amniotic fluid was clear and of a normal amount. Internal monitoring demonstrated a saltatory sinusoidal pattern with decreased variability (Fig. 1, *B*). The results of two scalp pH examinations were 7.29 and 7.28. Because the scalp blood samples

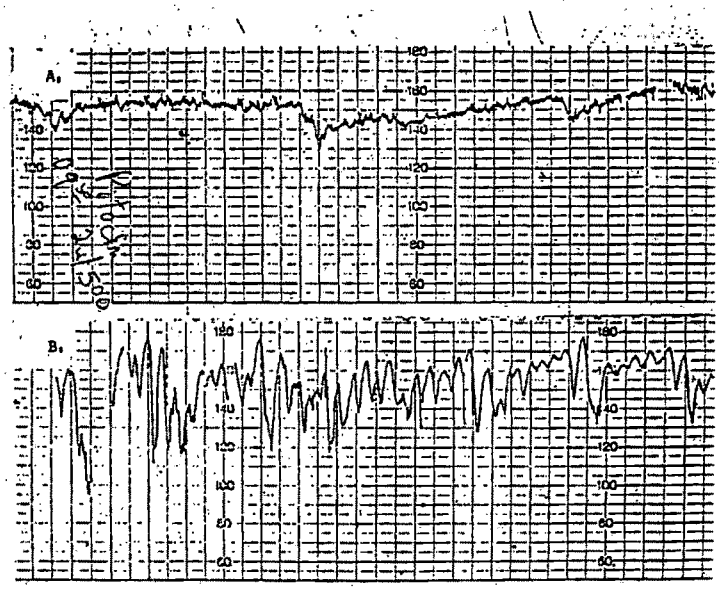


Fig. 1. Fetal heart rate recording. (Uterine contractions are not presented.) **A,** Upper recording. Positive oxytocin challenge test. **B,** Lower recording. Intrapartum internal monitoring.

seemed watery, scalp blood hematocrit level was determined. The result was 15% and an emergency cesarean section was performed. A pale, 3020 gm male infant with an Apgar score of 1 at 1 minute and 5 at 5 minutes, with cord blood pH of 7.25, was delivered. Immediate resuscitation included ventilation and blood transfusion. Initial hemoglobin was 3 gm/dl, hematocrit 10%, reticulocytes 3.8%, and negative hemolytic tests. Neonatal recovery was rapid with spontaneous breathing on the same day with normal astrope. The infant was discharged after 10 days without any abnormal finding.

Developmental examinations during the following year were normal. The peripheral maternal blood test for fetal red blood cells (Kleihauer and Betke) was 2.8% (approximately 150 ml of fetal blood) and established the diagnosis of fetomaternal hemorrhage. Meticulous pathologic examination of the placenta did not show any abnormality.

Comment

Intrapartum diagnosis of severe fetal anemia was described recently by Silva et al.[1] They used scalp hematocrit in a case of vaginal bleeding after amniotomy, and the low hematocrit level confirmed their suspicion of bleeding vasa previa.

Renaer et al.[2] described eight cases and reviewed the entity of fetomaternal hemorrhage as a cause of perinatal mortality and morbidity. In only one case was fetal anemia diagnosed antenatally in an alive fetus. They used maternal blood tests for fetal red blood count and neonatal blood after delivery. Those tests are recommended in any case of trauma, placental abruption, traumatic amniocentesis, external version, or unexplained fetal death.

None of these was valid in our case. In this case the nonpathologic scalp pH almost deceived us. Fortunately, the diluted appearance of the scalp sampling led us to examine fetal hematocrit. The test is feasible in most cases in which scalp sampling for pH is obtainable. We recommend its consideration in any case of suspected fetal bleeding, watery appearance of fetal blood, and significant discrepancy between fetal monitoring and scalp pH.

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Prevention of pelvic adhesion formation by different modalities of treatment

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This prospective study was designed to investigate the effect of glucocorticoids, promethazine (Phenergan), hyskon, heparin, and Ringer's lactated solution in the prevention of pelvic adhesion formation after pelvic surgery. The patients were divided into two groups: The first consisted of 396 patients who had laparotomy for different types of pelvic surgery, and the second group consisted of 546 patients who had operative laparoscopy for different types of pelvic disease. Patients in these two major groups were divided prospectively into different subgroups with different modalities of adjuvant therapy to prevent postoperative pelvic adhesions. A second-look laparoscopy was performed for some of the patients who failed to conceive in each of these subgroups. Pregnancy rate and adhesion formation were compared between these subgroups with no statistical difference noted. It is concluded that glucocorticoids, promethazine, heparin, and hyskon have no therapeutic advantage over Ringer's lactated solution in the prevention of postoperative pelvic adhesion formation. (Am J Obstet Gynecol 1987;157:1184-8.)

Key words: Adhesion formation, hyskon, promethazine, glucocorticoids, heparin, Ringer's lactated solution

The formation of postoperative pelvic adhesions remains a significant cause of failure in reconstructive surgery for restoration of fertility in women.[1, 2] Avoidance of serosal trauma by gentle tissue handling is thought to be the most important factor in preventing the formation of these pelvic adhesions.[2] Over the years, many drugs and other substances, including glucocorticoids, promethazine, heparin, and dextran, have been used to prevent or reduce the formation of postoperative adhesions.[3-5] Different studies recommended the use of different substances as adjuncts to pelvic surgery to improve the fertility outcome by preventing peritoneal adhesions.[5] Only a few of these studies have attempted to compare the effects of these adjuvants in a strictly controlled clinical manner.[3, 4] The present study was designed to provide a prospective and controlled evaluation of the effectiveness of glucocorticoids, promethazine, hyskon, heparin, and Ringer's lactated solution in the prevention of pelvic adhesion formation in women's infertility surgery.

Material and methods

Two groups of patients were prospectively studied to evaluate the effectiveness of certain drugs and solutions in preventing postoperative adhesions. The first group consisted of 396 women with primary or secondary infertility who had laparotomy for surgical correction of tubal, ovarian, uterine, or peritoneal abnormalities (Table I). One hundred forty of these women (subgroup I) received dexamethasone (Decadron), 20 mg intravenously, and promethazine (Phenergan), 25 mg intramuscularly 4 hours before surgery. The same dose was administered at the beginning of surgery and every 4 hours after surgery for 48 hours. The same dose of dexamethasone and promethazine was instilled into the pelvic cavity along with 200 ml of 32% dextran 70 (hyskon) before closure of the peritoneal cavity. Eighty-six women (subgroup II) received promethazine and hyskon in a manner similar to that used in subgroup I but without dexamethasone. Seventy-four

Table I. Types of procedures performed in the laparotomy group

Procedure	*I*	*II*	*III*	*IV*
Salpingoovariolysis	7	4	4	6
Fimbrioplasty	8	6	5	6
Salpingostomy	35	22	20	27
Midsegment anastomosis	32	20	17	22
Tubouterine anastomosis	18	9	10	12
Tubal implantation	12	4	3	4
Endometriosis excision	16	12	8	6
Myomectomy	8	5	4	8
Metroplasty	4	4	3	3
Total	140	86	74	96

I = Dexamethasone, phenergan, and hyskon; II = phenergan and hyskon; III = hyskon; IV = Ringer's lactated solution.

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Table II. Patients with laparotomy who had second-look laparoscopy

Procedure	*I*	*II*	*III*	*IV*
Salpingoovariolysis	2 (100%)*	1 (100%)	1 (100%)	2 (100%)
Fimbrioplasty	3 (67%)	2 (100%)	1 (100%)	2 (50%)
Salpingostomy	6 (67%)	4 (50%)	5 (60%)	6 (50%)
Midsegment anastomosis	8 (25%)	5 (20%)	4 (50%)	5 (40%)
Tubouterine anastomosis	4 (50%)	2 (100%)	3 (67%)	3 (67%)
Tubal implantation	3 (67%)	2 (100%)	1 (100%)	1 (100%)
Endometriosis excision	6 (100%)	6 (100%)	3 (100%)	3 (100%)
Myomectomy	2 (50%)	1 (0%)	1 (100%)	2 (0%)
Metroplasty	1 (100%)	1 (100%)	0	1 (100%)
Total	35	24	19	25

I = Dexamethasone, phenergan, and hyskon; II = phenergan and hyskon; III = hyskon; IV = Ringer's lactated solution.
*Parentheses indicated percentage of patients who developed postoperative adhesions.

women (subgroup III) had 200 ml of hyskon instilled into the pelvic cavity before abdominal closure but without any systemic or intraperitoneal medication. In 96 women (subgroup IV) only 400 ml of Ringer's lactated solution was instilled into the pelvic cavity before the parietal peritoneum was closed. The senior author was the main surgeon for all patients in whom microsurgical techniques were used when applicable, with great attention paid to gentle tissue handling. Earlier, patients who had endometriosis, usually of the moderate or severe type, were treated by laparotomy for resection, whereas patients who had no endometriomas were treated by operative laparoscopy regardless of the endometriosis stage. Later when we became more adept with operative laparoscopy, all stages of endometriosis were treated with this procedure. At laparoscopy, endometriosis was scraped or excised and endometriomas were drained and washed.[6,7] Because of the extended nature of this study, patients in the different subgroups who had the same operative procedure were relatively comparable. The study for this laparotomy group took 64 months to complete, with an allocation of 16 months for each subgroup starting with subgroup I and ending with subgroup IV. All patients received 200 mg of doxycycline intravenously at the beginning of surgery and 100 mg intravenously 8 hours after surgery. Doxycycline, 100 mg by mouth, was given twice a day for the first 5 postoperative days. A second-look laparoscopy was done for most of the patients who failed to achieve pregnancy in 9 to 12 months after surgery (Table II).

The second group consisted of 546 women with primary or secondary infertility who had operative laparoscopy for repair of possible pelvic disease (Table III). Patients were divided into three subgroups: The first consisted of 142 women who had 200 ml of hyskon instilled into the pelvis at the conclusion of the operative laparoscopy. The second group consisted of 174 women who had 400 ml of Ringer's lactated solution mixed with 1 gm of hydrocortisone and 2500 U of heparin instilled into the pelvis. The third subgroup consisted of 230 women who had Ringer's lactated solution only instilled into the pelvis at the conclusion of the laparoscopy. The study for this laparoscopy group took 54 months to complete, with 18 months allocated for each of the three subgroups. A second-look laparoscopy was performed for all patients who had severe endometriosis and for most patients who had moderate endometriosis after the completion of their Danazol treatment, which varied from 3 to 6 months. Patients who had undergone other operative laparoscopic procedures but failed to achieve pregnancy in 9 to 12 months received second-look laparoscopy (Table IV). The classification of endometriosis was based on the one adopted and recently revised by the American Fertility Society. It is important to mention that we did not try to assign a subgroup either in the laparotomy or laparoscopy group in whom only strict, meticulous microsurgery would have been performed without any of these adjuvants added because past experience repeatedly taught us that fluid in the peritoneal cavity after surgery is essential for its hydroflotation effect and to inhibit coagulation of the immediately released exudate.

Table III. Types of procedures performed in the laparoscopy group

Procedure	*I*	*II*	*III*
Salpingoovariolysis	32	42	59
Pelvilysis*	24	28	29
Fimbrioplasty	14	16	20
Endometriosis (MM) excision	41	49	70
Endometriosis (M) excision	21	25	30
Endometriosis (S) excision	10	14	22
Total	142	174	230

I = Hyskon; II = Ringer's lactated solution, hydrocortisone, and heparin; III = Ringer's lactated solution; MM = minimal to mild; M = moderate; S = severe.
*Lysis of pelvic adhesions not involving adnexa.

Table IV. Patients with laparoscopy who had a second-look laparoscopy

Procedure	*I*	*II*	*III*
Salpingoovariolysis	6 (16%)*	8 (12%)	10 (10%)
Pelvilysis	4 (0%)	4 (0%)	2 (0%)
Fimbrioplasty	2 (0%)	3 (0%)	3 (0%)
Endometriosis (MM) excision	3 (0%)	5 (0%)	7 (0%)
Endometriosis (M) excision	5 (40%)	5 (20%)	10 (20%)
Endometriosis (S) excision	7 (59%)	9 (55%)	14 (57%)
Total	27	34	46

I = Hyskon; II = Ringer's lactated solution, hydrocortisone, and heparin; III = Ringer's lactated solution; MM = minimal to mild; M = moderate; S = severe.

*Parentheses indicate percentage of patients who developed postoperative adhesions.

Table V. Pregnancy rate (%) in the laparotomy subgroups

Procedure	*I*	*II*	*III*	*IV*
Salpingoovariolysis	70	50	50	67
Fimbrioplasty	63	50	60	67
Salpingostomy	40	45	45	44
Midsegment anastomosis	66	65	60	63
Tubouterine anastomosis	55	55	60	74
Tubal implantation	25	50	34	50
Endometriosis excision	25	25	37	34
Myomectomy	63	80	75	63
Metroplasty	75	75	100	80

I = Dexamethasone, phenergan, and hyskon; II = phenergan and hyskon; III = hyskon; IV = Ringer's lactated solution.

Table VI. Pregnancy rate (%) in the laparoscopy subgroups

Procedure	*I*	*II*	*III*
Salpingoovariolysis	75	70	8[illegible]
Pelvilysis	83	73	80
Fimbrioplasty	50	62	60
Endometriosis (MM) excision	70	70	76
Endometriosis (M) excision	47	44	50
Endometriosis (S) excision	30	35	36

I = Hyskon; II = Ringer's lactated solution, and heparin; III = Ringer's lacated solution; MM = minimal to mild; M = moderate; S = severe.

Scoring of adhesions. Description of the sites and extent of uterine, adnexal, and general peritoneal adhesions were made at the time of the first surgical procedure and again at the time of the second-look laparoscopy. The uterus, cul-de-sac, and each tube and ovary were individually scored as 0 for no adhesions, 2 if one third of each of the uterus, tube, or ovary was involved by flimsy adhesions, 4 if two thirds were involved, and 6 if the whole organ was involved. If the adhesions were thick, each of these scores was multiplied by 2. A score of 5 was added if the cul-de-sac was obliterated by flimsy adhesions and 10 if obliterated by thick adhesions.

Results

Table V shows that there was no statistical difference in pregnancy rate between similar procedures for each of the four laparotomy subgroups (χ^2 test, $p > 0.777$). Table II shows the number of patients who had a second-look laparoscopy in each of the four laparotomy subgroups. There was no statistical difference in the rate and quality (median adhesion score) of adhesions developed for each specific procedure in the four subgroups (χ^2 test, $p > 0.733$).

Table VI shows that the pregnancy rate was almost similar for patients who had the same laparoscopic procedure performed, regardless of the subgroup to which they belonged. Table IV shows that no adhesions developed in patients who had pelvilysis, fimbrioplasty, or scraping of mild endometriosis in all three laparoscopic subgroups. No statistical difference was found in the rate or quality of adhesions developed or reformed in the remaining four procedures, regardless of the type of adjuvants used (χ^2 test, $p > 0.806$).

Comment

Movement of the tubes and probably the ovaries is essential for ovum pickup.[8] Because peritubal and periovarian adhesions limit this movement, they are considered a major factor in female infertility.[9] The meticulous reconstructive surgery of the infertility surgeon can be undone by the formation of postoperative adhesions. These adhesions occur after an inflammatory response caused by surgical trauma, anoxia, and foreign bodies such as suture material, bleeding, and infection. The prevention and treatment of pelvic adhesions have been an area of intense interest for infertility surgeons for many years. Intraperitoneal adjuncts and systematically administered drugs, including steroids, antihistamines, dextran, anticoagulants, antiprostaglandins, and antibiotics, were used and studied.[5] None of these agents has been unequivocally successful or unanimously accepted by infertility surgeons, since their effectiveness in preventing postoperative adhe-

sions is still controversial at best. The data in this report show that none of these adjuvants has an advantage over the inexpensive and complication-free Ringer's lactated solution in the prevention of postoperative pelvic adhesions.

An insult to the serosal surface results in the outpouring of fibrinogen, which is changed into a fibrin clot causing the adherence of adjacent structures. This fibrinous attachment then becomes progressively organized into a thick and vascularized fibrous adhesion. In an attempt to prevent the formation of adhesions after laparotomy, we tried four different modalities of treatment using dexamethasone, promethazine, dextran, and Ringer's lactated solution. In theory, dexamethasone stabilizes the cell membrane, decreases histamine release, and antagonizes its effect. In addition, it impedes fibroblast migration and proliferation and collagenous organization. Promethazine decreases histamine release, inhibits the increase in vascular permeability induced by histamine, and protects the cellular lysosome system.[10] The use of intramuscular and intraperitoneal dexamethasone and promethazine to prevent postoperative adhesions was originally described by Replogle et al.[11] These authors and others[12] subsequently promulgated such a systemic and intraperitoneal regimen in abdominal and infertility surgery. This present study and others[13] did not show any significant difference in the frequency or degree of adhesion formation or reformation after surgery between patients receiving dextran, crystalloid solutions, or a combination of dexamethasone and promethazine.

Hyskon, which is a concentrated (32%) high molecular–weight dextran (70,000), became popular subsequent to a report by DiZerega and Hodgen[11] of its apparent efficacy in a controlled study undertaken in four groups of monkeys with surgically induced abrasions. Dextran's mechanism of action remains to be proved. Theories suggest that it has a siliconizing effect and coats raw serosal surfaces and abrasions that prevent fibrinous adhesions. Probably the most important action of intraperitoneal hyskon is its hydroflotation effect that prevents opposition of surfaces. It is slowly absorbed and may stay in the peritoneal cavity as long as 2 weeks, bringing more fluid and thus prolonging its hydroflotation effect.[4] The present study, like previous ones, provides no support for its use because there was no difference in the quality and quantity of adhesion formation between patients treated with hyskon and those treated with Ringer's lactated solution.[3]

Heparin administered systemically or intraperitoneally was reported to be effective in inhibiting adhesion formation or reformation after surgery.[15] Optimal effectiveness was noted when it was administered in relatively high doses and for at least 3 days after surgery. The attendant risk of hemorrhage, as documented in laboratory and clinical studies, forced investigators to abandon heparin use for that purpose until recently.[16] The present study does not show any advantage with the use of intraperitoneal heparin, and therefore we do not recommend its use for the prevention of adhesion formation after surgery. In all laparoscopy subgroups, hyskon, hydrocortisone, and heparin proved to have no advantage over plain Ringer's lactated solution in the prevention of postoperative pelvic adhesion formation.

Over the years, we have kept all tissues exposed in the operative field constantly moist during both microsurgery and macrosurgery. We started with normal saline solution, but this was replaced by Ringer's lactated solution after we noticed that tissues irrigated with normal saline solution for more than 30 minutes blanch and swell and probably sustain minor serosal damage. Continuous irrigation with Ringer's lactated solution prevents fibrin deposition in the peritoneal cavity and washes away fibrinous exudate released from the cut tissue surfaces. The instillation of 400 to 500 ml of Ringer's lactated solution in the cul-de-sac will inhibit coagulation of the released exudate and keep serosal surfaces separated, thereby preventing the formation of fibrinous bridges and later fibrous adhesions. Most female pelvic cavities can accommodate this amount of fluid without any leakage through the incision.

Because infection results in tissue scarring and adhesion formation, almost all infertility surgeons gives some type of known broad-spectrum antibiotics prophylactically. Since antiseptic solutions instilled locally into the pelvis have been found to significantly reduce adhesion formation, there may be some merit to the use of prophylactic antibiotics after reconstructive pelvis surgery.[17] We did not encounter any postoperative pelvic infection probably because we routinely used doxycycline prophylactically. The extended 5-day course of prophylactic antibiotics was not based on scientific data; rather, it was our long-standing routine that never failed, and therefore we became dependent on it.

In summary, this study shows that postoperative adhesion formation can be minimized or even eliminated with gentle tissue handling and if raw surface areas are kept separated for 1 to 2 days after surgery. Plain Ringer's lactated solution seems to do the job, and therefore there is no need for the administration of other agents used in this study. Use of these agents is unnecessary and could prove to be dangerous because of the many side effects they may produce.[5]

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A thirty-year review of maternal mortality in Oklahoma, 1950 through 1979

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Oklahoma's Maternal Mortality Committee has been active since 1941. During the 30-year period 1950 through 1979, the committee reviewed in detail 75.9% of the pregnancy-related deaths that occurred in Oklahoma. The maternal mortality ratio in 1950 was 95.1/100,000 live births, and for 1979 it was 8.1/100,000 live births, a decrease of 91.5%. The risk of death from childbearing remained greater for black women than for American Indian or white women throughout the three decades. For American Indian women, the risk of death associated with pregnancy has decreased and is almost equal to the risk for white women. The Maternal Mortality Committee estimated that two thirds of Oklahoma's maternal deaths were preventable. The proportion of deaths judged preventable did not vary substantially during the study period. We conclude that maternal mortality in Oklahoma can be reduced to fewer than three deaths per 100,000 live births. Intensive monitoring and investigation of deaths and their causes by local maternal mortality committees continues to be an important mechanism for obtaining information to assist health workers in the prevention of deaths. (Am J Obstet Gynecol 1987;157:1189-94.)

Key words: Maternal mortality, Oklahoma, race-specific rates, Native American maternal mortality

The prevention of maternal deaths remains one of the foremost goals of obstetrics, and the maternal mortality ratio has been a widely used measure of the quality of maternal health care. We report here a descriptive epidemiologic study performed on the reported pregnancy-related deaths occurring over 30 years in the State of Oklahoma from 1950 through 1979. This included the analysis of maternal mortality ratios by age, causes of death, and trends over time in three racial groupings—white, black, and American Indian. Preventability factors were also studied.

Material and methods

The Oklahoma Maternal Mortality Committee was organized in 1941[1] by the Oklahoma State Medical Association, in cooperation with the Maternal and Child Health Service (MCHS) of the Oklahoma State Department of Health. Pregnancy-associated deaths were identified by Oklahoma vital statistics from death certificates and sent to MCHS. In order to learn more about the deaths, the committee requested additional information from the physician who had signed the death certificate and from the physician who had treated the mother. All identifying information was removed from the collected information and sent to the committee. After review by the committee, the deaths were classified according to the *Guide for Maternal Death Studies* published by the American Medical Association (AMA) in 1964.[2]

Causes of death have been reclassified by the codes given in the *International Classification of Diseases*, ninth revision, Clinical Modifications (ICD-9-CM), volume 1, 1979.[3] This edition was selected because it has a larger number of pregnancy-related causes of death than previous revisions of the ICD (namely revisions 6, 7, and 8, which were in use during the study period), and the ninth edition is the one in current use. The ICD-9-CM differs from the ICDA-8 and the AMA's classification[2] of maternal deaths in the following respects:

1. ICD-9-CM includes other concurrent conditions in the mothers, such as diabetes and cardiovascular diseases that complicate pregnancy, childbirth, and the puerperium. The AMA defined deaths due to these conditions as indirect obstetric deaths, while ICDA-8 classified them as deaths due to non–pregnancy-related causes.

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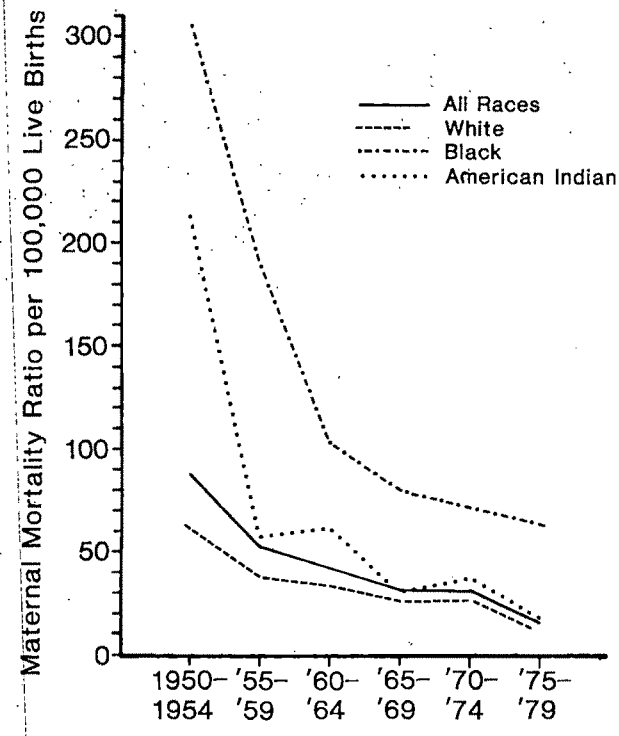


Fig. 1. Maternal mortality ratio by race and by 5-year intervals, Oklahoma, 1950 through 1979.

2. ICD-9-CM includes deaths from maternal infection and parasitic conditions such as venereal diseases and tuberculosis. These conditions are included in the AMA's definition of nonrelated deaths. These conditions are not included in the pregnancy-related causes of deaths in ICDA-8.
3. ICD-9-CM limits maternal deaths to those occurring within 42 days of termination of pregnancy, while ICDA-8 has a time period of up to 1 year after pregnancy termination and the AMA uses a time period of 90 days after pregnancy termination. In ICD-9-CM, deaths that were classified under codes 630-676 were called maternal deaths.

The maternal mortality ratio has been calculated with maternal deaths as the numerator and live births used as the denominator.[4] Thus the maternal mortality ratio is the number of maternal deaths per 100,000 live births. Because Oklahoma's racial groups differ in the distribution of maternal ages, a crude maternal mortality ratio and an age-adjusted maternal mortality ratio was calculated for each racial group, with the aggregate maternal age distribution during 1950 through 1979 as the standard population.[5] The Maternal Mortality Committee, using the *Guide for Maternal Death Studies* published by the AMA,[2] determined whether these maternal deaths could have been prevented. The Maternal Mortality Committee also attempted to establish whether the primary responsibility for these maternal deaths could be attributed to the attending physician, to the patient, or to both.

Results

A total of 632 maternal deaths among Oklahoma residents were reported to the Maternal Mortality Committee during the 30-year period from 1950 through 1979. In addition, the committee received reports of 61 pregnant women who died of causes unrelated to pregnancy, such as malignancies and automobile accidents. These latter deaths were excluded from the analysis. There were 1,405,382 live births in Oklahoma during the 30-year period. Thus the maternal mortality ratio in Oklahoma for 1950 through 1979 was 45.1 per 100,000 live births. The maternal mortality ratio in 1950 was 95.1 per 100,000 live births, and in 1979 it was 8.1 per 100,000 live births. The maternal mortality ratio decreased in the 1950s by 40.4% and in the 1970s by 49.2% (Fig. 1).

The age-specific maternal mortality ratio decreased for each age group during the three decades (Table I). During the 1950s, the age-specific maternal mortality rates were slightly higher for women 15 to 19 years old than those 20 to 24 years old and were highest for older women. The mortality ratio among teenagers declined more dramatically than the ratio for women aged 20 to 24, so that after the 1950s the maternal mortality ratio increased directly with increasing age.

Of the 632 deaths, 64.2% occurred among white women, 27.1% among black women, and 7.2% among American Indian women. Black women had the highest maternal mortality ratio throughout the study period (Fig. 1). The age-adjusted maternal mortality ratio decreased among white and American Indian women during the three decades. Black women showed a more rapid reduction in the mortality ratio between the first two decades, but the age-adjusted maternal mortality ratio for black women actually increased in the 1970s (Table II).

To compare maternal mortality ratios in the three racial groups, we divided two race-specific maternal mortality ratios to obtain a ratio of ratios or a "risk ratio." Thus for pregnant black women the risk of pregnancy-related death was 4.6 times greater than the risk for white women during the first decade of the study; this ratio decreased in the 1960s (2.7) but increased in the last decade (4.8). On the other hand, American Indian women's risk of pregnancy-related death was 3.2 times greater than the risk for white women during the 1950s, but this risk ratio steadily declined to 1.2 in the 1970s (Table II).

The proportion of maternal deaths attributed to hemorrhage, preeclampsia-eclampsia, and abortion complications (which were the three leading causes of maternal mortality) declined from 1950 through 1979,

Table I. Number of maternal deaths and maternal mortality ratio per 100,000 live births by age and decade, Oklahoma, 1950 through 1979

Age group (yr)	Total (1950-1979)		1950-1959		1960-1969		1970-1979	
	Ratio	No. of deaths	Ratio	No. of deaths	Ratio	No. of deaths	Ratio	No. of deaths
15-19	26.6	75	46.7	41	19.0	19*	15.3	15
20-24	25.6	135	34.2	61	22.7	40	19.7	34
25-29	46.2	156	70.8	87	39.6	40	25.5	29
30-34	71.2	114	105.9	74	56.7	28	29.5	12
35-39	133.5	96	166.1	58	114.0	28	80.6	10
≥40	253.4	55	334.2	36	206.9	16	95.5	3
Total	45.1	631	65.2	357	37.7	171	25.6	103

*Includes one 14-year-old girl in 1967.

Table II. Age-adjusted maternal mortality ratio per 100,000 live births by race, decade, and risk ratio, Oklahoma, 1950 to 1979

	Total (1950-1979)		1950-1959		1960-1969		1970-1979	
	Ratio	No. of deaths	Ratio	No. of deaths	Ratio	No. of deaths	Ratio	No. of deaths
Race								
All races	45.1	632	65.2	357	37.7	172	25.6	103
White	34.1	406	46.4	220	31.0	138	19.3	68
Black	129.5	171	214.3	102	83.2	40	92.6	29
Indian	67.4	55	144.3	15	53.7	54	22.7	6
Risk ratio								
Black/white	3.8		4.6		2.7		4.8	
Indian/white	2.0		3.2		1.7		1.2	

while the proportion of deaths due to puerperal infections and ectopic pregnancy increased (Table III).

Of the 632 maternal deaths, the marital status of 590 women was known (93.3%). Among deaths of women with known marital status, a total of 86.4% of maternal deaths occurred among married women. In the 1950s 89% of maternal deaths occurred among married women, while in the 1970s this percentage declined to 80.4%. Data on births by marital status were not available to calculate a maternal mortality ratio by mother's marital status.

Throughout the period, autopsies were performed in 203 maternal deaths in Oklahoma. The proportion of deaths with autopsies reported increased from 11.3% in 1950 to 1954 to 57.1% in 1975 to 1979. Similar increases occurred among all racial groups.

Although the total number of deaths among women of reproductive age decreased by 14% between 1950 to 1954 and 1975 to 1979, the proportion of maternal deaths decreased even more. Thus in 1950 to 1954 maternal deaths represented 6.5% of all deaths among women aged 15 to 44 while in 1975 to 1979 maternal deaths represented only 1.2%, a relative decrease of 81.5% (data not shown). This decrease occurred throughout the period among white and American Indian women; however, among black women, a slight increase occurred in the last 5 years of the study.

The Oklahoma Maternal Mortality Committee assessed preventability and responsibility factors in 75.0% of the maternal deaths. Throughout the study period, preventable factors were identified for 66.9% of those evaluated. The number of maternal deaths that had preventable factors identified decreased from 105 in 1950 to 1954 to 25 in 1974 to 1979, a relative decrease of 76.2% (Table IV), while the total number of maternal deaths decreased from 224 in 1950 to 1954 to 36 in 1974 to 1979, a relative decrease of 83.9%. The committee attempted to establish whether the primary responsibility for these maternal deaths was attributable to physician, patient, or both. They found that physicians were responsible for factors that could have been prevented in 52.1% of the assessed maternal deaths. Patients were responsible for factors that could have been prevented in 33.8% of the assessed deaths, and the physicians and patients combined were responsible for preventable factors in 11.7% of the maternal deaths. The percentage of maternal deaths with preventable factors that were the responsibility of the physician increased from 41.9% in 1950 to 72.2% in 1965, then declined to 40% in 1975 to 1979. In the same period,

Table III. Causes of maternal deaths by decade and race, Oklahoma, 1950 through 1979 (percentage distribution)

Cause	Total (1950-1979)	All races			Race-specific (1950-1979)		
		1950-1959	1960-1969	1970-1979	White	Black	Indian
Hemorrhage	24.4	27.2	22.1	18.5	24.9	21.0	30.9
Preeclampsia-eclampsia	17.1	20.5	12.8	12.6	15.3	19.9	21.8
Complications of abortion	8.7	8.7	9.3	7.8	8.1	9.4	10.9
Puerperal infection	7.2	3.5	9.9	15.5	7.4	4.1	16.4
Ectopic pregnancy	7.0	6.2	5.8	11.7	5.2	12.9	1.8
Amniotic fluid embolism	4.3	1.7	8.7	5.8	5.2	2.3	3.6
Pulmonary embolism	4.3	4.2	5.8	1.9	5.7	1.8	1.8
Complications of anesthesia	5.2	4.8	6.4	4.9	4.4	8.2	1.8
Cardiovascular disorder	7.0	8.7	5.2	3.9	7.6	6.4	3.6
Others	14.8	14.5	14.0	17.4	16.2	14.0	7.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
No. of deaths	632	357	172	103	406	171	55

Table IV. Responsibility for preventable factors resulting in maternal deaths assessed by Maternal Mortality Committee, Oklahoma, 1950 through 1979 (percentage distribution)

Responsibility	Years					
	1950-1954	1955-1959	1960-1964	1965-1969	1970-1974	1975-1979
Physician	41.9	50.4	59.6	72.2	61.5	40.0
Patient	40.0	34.0	25.4	16.7	28.2	56.0
Physician and patient	15.2	2.4	12.8	11.1	5.2	4.0
Other	2.9	3.2	2.2	0.0	5.1	0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0
Estimated No. of preventable deaths	105	65	47	36	39	25

the percentage of maternal deaths with preventable factors that were the responsibility of the patient decreased gradually from 40.0% in 1954 to 16.7% in 1965 to 1966, then climbed up to 56.0% in 1975 to 1979.

Comment

The decline of maternal mortality in Oklahoma closely parallels that of the United States as a whole. Oklahoma's maternal mortality ratio, as calculated by the National Center for Health Statistics, was 85.2/100,000 live births in 1950 and 10.1/100,000 live births in 1979, a decrease of 88.2%. During the same period, U.S. maternal mortality ratios decreased by 90.6%.

Studies in several states found that the incidence of maternal mortality was higher than that reported through the standard vital records reporting systems.[6-9] The method of ascertaining maternal deaths through maternal mortality committee review may be more complete than using national vital statistics alone.[8] The rates observed in this study are higher than those obtained from the National Center for Health Statistics. These studies have underscored the importance of improved surveillance in identifying maternal deaths. The maternal mortality committee is one way of clarifying the cause of maternal deaths.

Many reported studies calculated the maternal mortality ratio with direct obstetric death as defined by the guidelines set by the AMA used as the numerator.[9, 10] Rochat[12] emphasized that if pregnancy-related deaths were to be used as the numerator instead of direct obstetric deaths, the ratio may be twice as high as the current reported maternal mortality ratio. ICD-9-CM comes closer to measuring pregnancy-related deaths than the AMA classification or previous revisions of the ICD. For the 30-year period of this study, use of the ICD-9-CM classification resulted in 23.8% more deaths classified as maternal deaths when compared with those classified by the AMA's direct obstetric death classification.

Smith et al.[13] classified all maternal deaths in the United States from 1974 to 1978 by using ICD-9-CM and ICDA-8 and found that the use of ICD-9-CM resulted in an increase of 8.8% over ICDA-8 in the number of deaths classified as maternal deaths. All maternal deaths identified in this study occurred less than 42

days after delivery and met the ICD-9-CM definition of maternal death.

The race-specific analysis revealed that among black and American Indian women, maternal mortality ratios are still higher than the ratio for white women. The risk of death from childbearing remained greater for black women, while for American Indian women, the risk of death due to pregnancy has decreased and is similar to that of white women. The improvement in the American Indian maternal mortality ratio in Oklahoma may reflect improvements in obstetric care, improvements in socio-economic status, or an artifactual difference in reporting racial status on birth or death certificates. The important observation from the perspective of health care services is that the difference between ratios for black women and those for white women did not decrease. In fact, this difference has increased in the last decade. This difference is due in part to the increased proportion of maternal deaths of black women that are due to ectopic pregnancy and preeclampsia-eclampsia.

The percentage of deaths due to maternal causes among black women of reproductive age was higher than the percentage of deaths due to maternal causes among American Indian and white women. Factors contributing to this elevated risk are that black teenagers have a higher fertility rate than white teenagers in Oklahoma[14] and that black women may wait until later in their pregnancies than American Indian or white women to seek prenatal and obstetric care.

The study of Buehler et al.[15] revealed that black women aged 35 or older are at significantly higher risk of death than white women and their risk was even greater for deaths associated with abortive outcomes (including ectopic pregnancy). A significant finding was the higher rate of deaths in ectopic pregnancy among older black women. This reflects the fourfold higher rate of ectopic pregnancies among black women aged 35 and older compared with white women in the United States. One contributing factor to death from ectopic pregnancy is a failure to diagnose early pregnancy among women aged 35 and older. The diagnosis of ectopic pregnancy by physical examination is more difficult in these women because a greater proportion are overweight.[16]

Progress has been made in reducing the number of maternal deaths in Oklahoma, especially those due to hemorrhage, preeclampsia-eclampsia, and complications of abortion. In 1965, Crosby[17] reported that hemorrhage was the leading cause of maternal deaths, and a later study by Jimerson and Crosby[18] showed that hemorrhage continued to be the leading cause of maternal death in Oklahoma. More recently, hemorrhage has dropped behind preeclampsia-eclampsia as the leading cause of maternal deaths.[19]

The number of maternal deaths due to preeclampsia-eclampsia fell precipitately between the 1950s and the 1960s. Tyler and Saeger[20] reported that preeclampsia-eclampsia was the leading cause of pregnancy-related deaths among American Indian women in eastern Oklahoma in 1965. Vaughn[21] reported that 30% of total maternal deaths among American Indians in New Mexico were due to preeclampsia-eclampsia. Our study also showed a similar pattern among American Indians in Oklahoma. Pregnant American Indian women are two and a half times more likely to die of preeclampsia-eclampsia than are white women. Early identification of high-risk women may well prevent maternal mortality caused by preeclampsia-eclampsia.[20]

Although complications of abortion were the third leading cause of maternal deaths in Oklahoma over the entire period, there were no deaths due to complications of induced abortion during the last 5 years. This may be due to widespread use of effective methods of contraception and to the availability of safe, legal abortion in Oklahoma.

Among all maternal deaths, the percentage of women who were single has more than doubled in the 30-year period; this phenomenon is particularly notable among black women. These deaths may reflect the trend of more births occurring out of wedlock in recent years and the higher percentage of out-of-wedlock births among black teenage women than among American Indian or white women.[14]

Since preventable factors were identified in two thirds of the maternal deaths evaluated, maternal mortality in Oklahoma could be reduced to three deaths per 100,000 live births. However, achieving this residual reduction in mortality will require intensive efforts to understand the events leading to maternal death.

Clinical data are particularly useful in determining the evaluation of events that led to the woman's death, but clinical data alone do not always provide adequate information to accurately classify the cause of death. Autopsies provide useful additional information. Although the proportion of maternal deaths evaluated with autopsies increased over the three decades studied, clinicians should continue to strive to obtain permission for autopsies to determine the specific pathologic causes of maternal death.

Although the recent dramatic decrease in Oklahoma's maternal mortality is encouraging, continued effort in mortality surveillance and health education will be necessary to meet these objectives.

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Anorexia nervosa, bulimia, and pregnancy

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Of 74 women previously treated for anorexia nervosa or bulimia, 15 had conceived 23 pregnancies when assessed at follow-up. The status of the eating disorder, course of pregnancy and delivery, infant health, and postpartum adjustment are described. Women in whom eating disorders were in remission at conception had greater maternal weight gain and babies with higher birth weights and 5-minute Apgar scores than women who conceived while they still had symptoms of restricting anorexia nervosa or bulimia. Women who had symptoms of eating disorders at conception also had continuance or worsening of these symptoms during pregnancy and the postpartum year. We recommend delay of pregnancy until the eating disorder is truly in remission. (Am J Obstet Gynecol 1987;157:1194-8.)

Key words: Anorexia nervosa, bulimia, pregnancy, infants

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The high prevalence of anorexia nervosa and bulimia among young women,[1] combined with more effective treatment of these conditions, has resulted in more of these women eventually becoming pregnant. Little is known about the course of pregnancy in these women and even less is known about the health of their infants. Many psychological conflicts thought to be important in patients with eating disorders concerning adult sexuality, body image, autonomy, dependency, and relationships to parents are highlighted during normal pregnancy. This has led to speculation that pregnancy

is especially stressful for women with eating disorders and that psychiatric difficulties, including exacerbation of anorexia nervosa and bulimia, are likely to occur during their pregnancy or postpartum period.

In reviewing the literature of the last 30 years, we found nine multiple case reports of three to 86 pregnancies occurring in women who had previously or concurrently suffered from anorexia nervosa.[2-10] Of these, only the study of Hart et al.[8] gave the periods of gestation and infant birth weights, and none gave the Apgar ratings of the infants. By far the largest study in this group by Brinch et al.,[10] published in Danish in 1985, reported 86 pregnancies occurring in 50 women who had been treated for anorexia nervosa during the previous 4 to 22 years. This study surprisingly reported seven perinatal deaths, with six attributed to prematurity. Unfortunately, weight gain in pregnancy, the length of gestation, infant weights, and Apgar scores were not reported for the study group. Seven single case reports published during the same period of time suffered from similar omissions. In the majority of these studies, many important factors, such as the status of the eating disorder, maternal weight gain, gestational period, psychological complications during and after pregnancy, infant birth weight, and Apgar ratings, were unreported.

It is the purpose of this study to report on the course of pregnancy and the health of the infants in a North American group of women treated for eating disorders, to determine the effects, if any, of anorexia nervosa and bulimia on their pregnancies, infants, and postpartum adjustment.

Material and methods

Seventy-four women treated for anorexia nervosa of the restricting or bulimic forms (according to the criteria of Garfinkel and Garner[11]) at a University of Toronto Eating Disorders Clinic between 1970 and 1978 were interviewed as part of a study of their long-term outcome.[12] Approval was obtained from the University of Toronto Review Committee on the Use of Human Subjects for the women to be contacted and interviewed and their charts reviewed to determine the course of any pregnancies that occurred after the diagnosis of the eating disorder. Of these women, 15 were identified as conceiving a total of 23 pregnancies. A variety of factors were identified in the 59 women who had not been pregnant; single status, infrequent intercourse, contraceptive use, and failure to conceive.

Information on those women who had had pregnancies after treatment for anorexia nervosa with or without bulimia was obtained from patient interviews and charts about four topic areas: the eating disorder, the course of pregnancy and delivery, the health of the infant, and the maternal complications including postpartum adjustment. These data are presented in Table I and are complete except for 5-minute Apgar scores for three healthy term babies with an average weight of 2722 gm, delivered vaginally of one mother in England.

The women were divided into three groups dependent on the status of the eating disorder at conception: patients with restricting anorexia nervosa who did not experience bulimia and who controlled weight primarily by caloric restriction; patients with bulimic anorexia who engaged in binge eating usually followed by self-induced vomiting or purging, and patients in remission without symptoms of eating disorder at conception.

The average age at first diagnosis of the eating disorder was 20 years for the women with restricting anorexia nervosa; 21 years for the bulimic women, and 18 years for the women in remission, with a range from 14 to 23 years. Eleven patients had been hospitalized from one to four times because of their eating disorder. The women with restricting anorexia and bulimic anorexia had been admitted an average of once per patient and the women in remission had been admitted an average of 1.5 times per patient.

Eight women who were asymptomatic at conception and in remission had 13 pregnancies; four women had active restricting anorexia and had seven pregnancies, and three women were binging, vomiting, and purging and had three pregnancies.

For purposes of statistical analysis all relevant information for these three groups was compared by use of one-way analysis of variance followed by Duncan's multiple range test for each of maternal weight gain, birth weight, and 5-minute Apgar scores.

Results

Pregnancy and delivery. All the women were married at the time of conception. The average age at conception was 27 years for those with bulimic anorexia and those in remission and 25 years for those with restricting anorexia. The average length of time from the initial diagnosis of eating disorder until conception was 9 years for the women in remission, 6 years for the bulimic group, and 5 years for the women with restricting anorexia. Weight gain in pregnancy varied from a loss of 8 pounds in a woman with restricting anorexia, S(12), who subsequently had a spontaneous abortion at 12 weeks, to a gain of 60 pounds in a previously bulimic woman, S(3), in remission. From Table II, it can be seen that those women in remission gained significantly more weight than those women with restricting anorexia nervosa and that the women with active bulimia gained even less ($p < 0.005$).

Of the 23 pregnancies conceived by the 15 women, 20 of them proceeded to live births; S(9) had an intrauterine death at 15 weeks, S(12) had a spontaneous abortion at 12 weeks, and S(7) underwent an elective abortion at 10 weeks.

Table I. Eating disorders, pregnancy, and infant factors

Subject identification	*Group**	*Age at first diagnosis (yr)*	*No. of hospitalizations*	*Pregnancy No.*	*Age at conception (yr)*	*Maternal weight gain (lbs)*	*Weeks' gestation*	*Infant birth weight (gm)*
S(1)	R	18	0	1	18	12	37	2762
	R			2	22	30	38	3997
S(2)	R	19	2	1	30	20	38	3487
	R			2	35	30	39	3856
	R			3	37	35	39	4479
S(3)	R	20	2	1	28	31	39	3657
	R			2	31	60	40	4224
S(4)	R	15	4	1	27	24	40	3629
S(5)	R	14	1	1	26	18	40	2778
S(6)	R	19	2	1	28	23	39	3260
S(7)	R	18	0	1	24	0	10	—
	R			2	26	25	40	3346
S(8)	R	17	1	1	25	28	40	3629
S(9)	A	23	0	1	26	4	15	—
	A				28	17	39	2410
S(10)	A	19	2	1	29	20	39	3147
S(11)	A	18	1	1	22	17	40	3175
	A			2	24	12	36	2268
	A			3	27	13	38	2722
S(12)	A	19	1	1	20	−8	12	—
S(13)	B	22	1	1	29	13	37	2552
S(14)	B	21	0	1	29	0	36	2098
S(15)	B	20	1	1	22	4	38	2438

*R = Remission; A = anorexia (restricting); B = bulimia.

Table II. Results of one-way analysis of variance in three groups of patients

	Remission (mean ± SD)	*Restricting anorexia*	*Bulimia*	*f Ratio*	*Level of statistical significance*
Maternal weight gain (lbs)*	28.0 ± 11.9	15.8 ± 3.3	5.7 ± 6.7	7.14	$p < 0.005$
Infant birth weight (gm)*	3592.0 ± 518.8	2744.4 ± 414.3	2362.7 ± 236.2	11.27	$p < 0.008$
Apgar score (5 min)†	8.8 ± 1.1	7.5 ± 2.1	4.0 ± 1	18.32	$p < 0.001$

*Significantly greater in remission group than in other groups (Duncan's test, $p < 0.05$).
†Significantly less in bulimic group than in other groups (Duncan's test, $p < 0.05$).

Of the 13 pregnancies that occurred in women in remission, only one had recurrence of eating disorder symptoms during pregnancy. The one patient, S(1), who encountered problems was a previously bulimic woman in remission, who started restricting food during her first pregnancy but was in remission throughout her second pregnancy 4 years later. In contrast, most patients who had symptoms of the eating disorder at conception reported worsening symptoms or psychological difficulty during pregnancy. Hospitalization for eating disorders during pregnancy occurred in two women, S(12), the woman with restricting anorexia who spontaneously aborted at 12 weeks, and S(14), who had bulimia. Both of these women had eating disorder symptoms at conception that seriously worsened during pregnancy.

In 18 of the 20 live births, the infants were delivered vaginally at 36 to 40 weeks' gestation. The average duration of gestation for term pregnancies was 37 weeks for the women with bulimia, 38 weeks for the women with restricting anorexia, and 39 weeks for women in remission. There were two cesarean sections, both in women with active bulimia: S(15), who had gained 4 pounds by 38 weeks, and S(14), who had not gained any weight by 36 weeks' gestation. In summary, all the complications of pregnancy and delivery occurred in women who were ill at conception.

Health of infants. The birth weights of the infants were significantly different among the three groups of women ($p < 0.008$). Women in remission had heavier babies in comparison with those of the women with restricting anorexia and bulimia (Table II). Infants of mothers in remission also had higher 5-minute Apgar scores when compared with mothers with restricting

5 min Apgar score	*Maternal/infant complications*
7	
9	
8	
9	
9	
8	
8	
10	
7	
10	
—	Elective abortion
10	
10	
—	Intrauterine death
6	
9	
—	
—	
—	
—	Hospitalized during pregnancy; spontaneous abortion
4	Respiratory distress
3	Hospitalized during pregnancy; cesarean section
	Jaundiced baby
5	Cesarean section

and bulimic forms of anorexia nervosa ($p < 0.001$) (Table II). As noted earlier, the two fetal deaths (a spontaneous abortion and an intrauterine death) occurred in women who were ill at conception. Only two infants were reported to have had difficulties after birth and both were born to bulimic mothers. The infant of S(13) weighed 2552 gm after spontaneous vaginal delivery at 37 weeks. The mother had gained 13 pounds during pregnancy. The infant had respiratory distress with a 5-minute Apgar score of 4. The infant of S(14) weighed 2098 gm, was jaundiced, and had a 5-minute Apgar score of 3. A cesarean section was performed at 36 weeks, and there had been no weight gained during pregnancy.

Postpartum adaptation. Six of the eight patients in remission at conception remained well for 1 year post partum. Of the two who had difficulty, S(2) developed a recurrence of anorexia nervosa shortly after her first delivery and has since developed an anxiety disorder after her third child. S(3) developed a major affective disorder 4 months post partum. In contrast, all seven women who were ill at conception continued to be ill or had worsening of the eating disorder in the postpartum year.

Of the 10 infants who were successfully breast-fed for 6 months or longer, nine were born to women in remission, one to a bulimic woman S(13), and none to women with restricting anorexia.

Comment

Despite the small numbers of women in the sample groups, some patterns emerged that have treatment implications. If the eating disorder is in remission, then an uncomplicated pregnancy and healthy baby can probably be expected. Our data for these women in remission do not support the Danish study by Brinch et al.[10] reporting high perinatal mortality rates in their infants, but further investigations are required. Especially important is the definition of remission of the eating disorder. In a related publication[12] we have shown that women who have had an eating disorder and are thought to be recovered have no more features of continued illness than a control group of women of comparable age. Women with active anorexia nervosa and bulimia at the time of conception had many difficulties, as shown in Table II; they gained less weight in pregnancy and had smaller babies with lower 5-minute Apgar scores. They also had frequent worsening of the eating disorder symptoms during pregnancy and the postpartum period, a finding similar to that recently reported by other investigators.[13] They also appear to have more difficulty with breast-feeding.

Some of these findings can be explained on the basis of the low weights of the actively ill women at the time of conception. A recent American study shows that pregravid body mass and pregnancy weight gain both significantly influence birth weight.[14] Literature from the Third World and Dutch famine suggest that the woman's weight-for-height before conception is as valid a predictor of maternal weight gain and infant weight as is nutritional status during pregnancy.[15] If this is so, then women with restricting anorexia and bulimia are at extra risk because they are underweight when they become pregnant and do not usually achieve adequate nutrition during pregnancy. It must be emphasized that some bulimic women may appear to be at a normal weight if one consults normative charts, but since they have been significantly overweight before becoming ill they may be undernourished relative to what is normal for them.[16]

At present it seems prudent to advise anorexic and bulimic women who wish to become pregnant to wait until the eating disorder is truly in remission, because the pregnancies and infants of such women may be at higher risk. We have similarly reported[17] that some women with eating disorders causing amenorrhea, who presented to an infertility clinic seeking hormonal induction of ovulation, had pregnancies complicated by the eating disorder and produced smaller babies with depressed Apgar scores. It would therefore seem wise for obstetricians to inquire about a history of eating disorder in infertile women and to counsel delay of pregnancy until the anorexic or bulimic symptoms have stopped. It would seem most helpful for the physician

to ensure that appropriate psychiatric treatment and follow-up are in place for the eating disorder rather than attempt to induce ovulation.

Physicians should also consider the possibility of an eating disorder in women who fail to gain appropriate weight during pregnancy or who present with intractable vomiting or purging. We recognize the need for further prospective studies with larger groups of women, looking at the course of the eating disorder during pregnancy and follow-up of their children.

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Effects of aerobic and strength conditioning on pregnancy outcomes

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This study evaluated the effects of a physical conditioning program on pregnancy outcomes and the subjective pregnancy experience. Pregnancy outcomes included length of labor, mode of delivery, length of hospitalization, and gestational age, birth weight, and Apgar scores of newborn infants. Subjective data included level of tension, general physical comfort, and self-image. The 845 subjects in the study were divided into a control group (n = 393) and into groups of low, medium, and high levels of exercise (n = 82, 309, and 61, respectively). Exercise sessions consisted of treadmill or bicycle warm-up, individually prescribed exercises on weight-lifting equipment for arms, legs, abdomen, and back, and 1- to 2-mile workout on bicycle ergometer. Pregnancy outcomes were more favorable in the exercise groups, particularly the high-exercise group. Significant data included length of hospitalization, incidence of cesarean section, and Apgar scores. All patients reported positive subjective responses to the conditioning program. (AM J OBSTET GYNECOL 1987;157:1199-203.)

Key words: Exercise during pregnancy; physical conditioning in pregnancy; strength conditioning in pregnancy; weight training in pregnancy; aerobic conditioning in pregnancy

An estimated 15 to 20 million women in the United States are actively involved in organized exercise programs.[1] Millions of others exercise on their own. Of these, an unknown number are pregnant or plan a pregnancy.

Pregnant women need a safe, efficient, and productive means of improving or maintaining muscular and cardiovascular strength and endurance throughout gestation. Their bodies may then meet the progressive physical demands of pregnancy in a more comfortable manner. Particularly, prescribed exercise enables their bodies to compensate for the anterior shift in the center of gravity as term approaches. Without compensation, this shift produces biomechanical changes that frequently cause physical discomfort and necessitate curtailment of activities.

Studies in the literature increasingly deal with the effects of aerobic exercise during pregnancy.[2-6] However, the cardiovascular component is only one of the enabling or limiting factors affecting prenatal, natal, and postpartum function. Equally important is the neuromuscular component, which has been virtually ignored in pregnancy for many reasons. To fill this unmet need, we designed the exercise protocol described in this report.

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The purpose of this study was to determine the effects of physical conditioning on both quantifiable pregnancy outcomes and the subjective experience of pregnancy. It was our hypothesis that the development of the muscular strength and/or flexibility needed to support the enlarging organs of the pregnant woman and improvement of her aerobic fitness have two main effects: (1) minimization of biomechanical changes during pregnancy and therefore lessening of prenatal discomfort and (2) minimization of labor and delivery complications. Prenatal comfort was assessed by subjective reports of the patient and included tension level, general physical comfort, and sense of well-being. Labor and delivery data included length of labor, type of delivery, length of hospital stay, and gestational age, birth weight, and 1- and 5-minute Apgar scores of the neonate. In addition, postnatal evaluation of the patient's labor and delivery experience and postpartum recovery was conducted.

Material and methods

Subjects. The subjects in the study consisted of 845 pregnant women who received obstetric care in private practice settings in Ocala, Florida. Subjects were referred to the exercise program by seven board-certified obstetricians. The women in the study were middle to upper middle class in socioeconomic status. Age and parity of the women appear in Table I.

Preliminary evaluation. An initial history was recorded and physical examination performed by each subject's obstetrician in the private practice setting. If no contraindications to exercise were identified, the

Table I. Age, parity, and pregnancy outcomes of women in the control group compared with low-, medium-, and high-exercise groups

Variable	*Control subjects (n = 393)*	*Exercise group*		
		Low (n = 82)	*Medium (n = 309)*	*High (n = 61)*
Age (yr)	30.0	26.2	24.5	25.2
Parity	0.9	0.8	0.6	0.7
Cesarean section (% of group)	28.1	23.1	19.0	6.7
Gestational age (wk)	39.9	40.1	40.1	40.4
Birth weight (gm)	3359	3471	3445	3510
1 min Apgar score	8.6	8.8	8.7	8.9
5 min Apgar score	9.3	9.1	9.5	9.6
Hospitalization (days)	2.9	2.6	2.5	2.2

subject was given the option to participate in an exercise program designed specifically for pregnant women. The exercise center at which this program was offered (Pregnagym) was located in a special facility adjacent to the offices of D.C.H.

At the exercise center, a detailed fitness evaluation was carried out by a physician specializing in exercise physiology. The evaluation included: (1) resting heart rate, (2) resting blood pressure, (3) standard physical therapy postural examination to identify lengthened and/or shortened muscle groups, (4) summation of skin-fold thickness in the triceps, subscapular, and suprailiac areas with a Lange skin-fold caliper, and (5) test of predicted submaximal oxygen uptake on a bicycle ergometer. In addition, a history was taken of past or present orthopedic, obstetric, or cardiopulmonary problems. The muscular and cardiopulmonary demands of the patient's vocational and recreational activities were also assessed. (This evaluation was repeated at 10-week intervals throughout the pregnancy.)

Exercise prescription. Historical findings and the results of all tests were used in determining the subject's exercise prescription. Exercise prescriptions had three components: (1) treadmill or bicycle warm-up, (2) use of selected exercise machines, and (3) aerobic workout on exercise bicycles.

The warm-up consisted of 5 minutes on a treadmill at a walk/jog rate of 3 to 4 mph or 5 minutes on an exercise bicycle at 300 kilopond meters (kpm).

After the warm-up period, the subject was assigned to workouts on exercise machines selected to strengthen and/or increase the flexibility of desired muscle groups of the upper and lower extremities and trunk on the basis of her evaluations. The amount of resistance on each machine was individually determined. If evaluation had revealed shortened muscle groups, the prescription was designed to lengthen these muscles by stressing the flexibility portion of the exercise without increased resistance. Progressive resistance was used for muscle groups that were found to be lengthened and needed strengthening. Once the subject was able to complete 12 repetitions in good form the resistance was adjusted on the basis of her individual capabilities. Total exercise duration averaged 45 minutes.

The final component of the prescription was an aerobic workout on one of various exercise bicycles. A resistance of approximately 300 kpm for 1 to 2 miles was prescribed for most women. Resistance was prescribed to accelerate heart rate to 85% of maximum (not to exceed 140 bpm).

Subjects were asked to exercise 3 days per week and were encouraged to continue training until the onset of labor or until a medical contraindication developed (this did not occur in any of the subjects). Individual training sessions were conducted by three full-time exercise instructors with special training in obstetric physiology and conditioning as well as the psychological aspects of pregnancy. Records of each exercise session were maintained.

Because of the progressive nature of the physical changes of pregnancy, repeat evaluations were scheduled every 10 weeks, and the subject's exercise prescription was modified accordingly. In addition, continuous maternal and fetal heart monitoring was performed, with a Litton 660 fetal monitor, throughout one session in the twenty-eighth week of gestation and another in the thirty-eighth week.

Study groups. Subjects were categorized into four groups, depending on the number of exercise sessions completed during the pregnancy. Control subjects (n = 393) had a mean of 0.8 sessions (range 0 to 10). The low-exercise group (n = 82) had a mean of 15 sessions (range 11 to 20). The medium-exercise group (n = 309) had a mean of 32 sessions (range 21 to 59). The high-exercise group (n = 61) had a mean of 64 sessions (range 60 to 99).

Data collection. The data were collected retrospectively from the hospital and exercise center records of the patients. Medical data included total labor time,

duration of the second stage of labor, incidence of cesarean section, length of hospital stay, gestational age of the neonate, birth weight, and 1- and 5-minute Apgar scores. At 6 weeks post partum, all participants were surveyed regarding their subjective responses to the exercise program: (1) self-image, (2) reduction in discomforts of pregnancy (for example, low back pain), (3) relief of tension, and (4) in multiparous patients, the rapidity of the recovery period compared with that of previous pregnancies.

Statistical treatment of the data regarding pregnancy outcome was done by simple analysis of variance and, when necessary, Duncan's multiple range test. A p level of 0.05 was selected to denote statistical significance.

Results

Mean lengths of labor in the study groups were calculated only for primigravid women, since multigravid women have shorter total labors and second stages of labor and there was a discrepancy in the representation of multigravid women among the groups. No significant differences in times of labor were found.

The rate of cesarean section was only 6.7% in the high-exercise group and 19.0% in the medium-exercise group, compared with 28.1% in control subjects and 23.1% in the low-exercise group ($p < 0.0001$) (Table I).

Birth weights of babies of control mothers averaged 65 to 151 gm below those of mothers in the three exercise groups (Table I), with the greatest contrast (151 gm) occurring between the control and high-exercise group ($p = 0.06$).

Babies of high-exercise mothers had slightly higher 1-minute Apgar scores (mean 8.9) than babies of mothers in the control group (8.6), low-exercise group (8.8), and medium-exercise group (8.7) ($p < 0.05$). The mean 5-minute Apgar scores for babies of mothers in the high-exercise group were also slightly higher than those for babies of mothers in the control and medium-exercise groups, that is, 9.6 versus 9.3 and 9.1 ($p < 0.05$) (Table I).

The mean hospital stay was slightly longer for women in the control group (2.9 days) than for those in all three exercise groups (range 2.2 to 2.6 days) ($p < 0.0001$), despite the fact that the mean parity was higher in the control group (Table I).

Maternal and fetal heart rates were monitored during one exercise session in the twenty-eighth week of gestation and another in the thirty-eighth week, for women who proceeded that far in the conditioning program. Maternal heart rates were appropriately elevated during exercise, while fetal heart rates remained normal in all cases. At no time was fetal bradycardia observed as a result of the aerobic or anaerobic exercise stimulus.

When participants in the study were surveyed at 6 weeks post partum regarding their subjective responses to the exercise sessions, all reported that the conditioning program gave them an improved self-image as well as a decrease in the common discomforts of pregnancy as long as they participated. Those who stopped the program for more than 2 weeks had a return of many of their aches and pains (especially low-back pain) and self-image declined. All patients stated that the program relieved tension. Patients who continued their conditioning until the onset of labor thought the exercise was beneficial during labor and delivery. It was the impression of the multiparous patients that their recovery period was more rapid than that after previous pregnancies.

Comment

Strenuous exercise has many potential dangers during pregnancy, for example, hyperthermia, dehydration, hypoxia, "energy drain," and possible injury to the expectant mother and/or developing fetus. It is not surprising then that the practicing obstetrician in this litigious society tends to be conservative about prescribing exercise regimens. Of particular concern to most obstetricians is undetected fetal hypoxia.

One cause of fetal hypoxia could be excessive catecholamine production associated with maternal exercise stress. Rauramo et al.[7] have shown that pregnant women respond to induced exercise with signficant increases in both epinephrine and norepinephrine. In experiments on rhesus monkeys, Adamsons et al.[8] demonstrated that either epinephrine or norepinephrine can produce severe asphyxia of the fetus when given in amounts that elicit a distinct increase in maternal blood pressure. However, physical conditioning can decrease the catecholamine response to a given exercise stress.[9] Therefore it may be potentially beneficial to condition the expectant mother before the onset of the greatest physical stress she and her fetus will experience during the course of the pregnancy—labor and delivery. Animal studies suggest that such conditioning is beneficial; blood diversion away from the uterus in the pregnant sheep and guinea pig is less when these animals have undergone physical training.[10, 11]

Our conditioning program was designed in part to control catecholamine production and thereby minimize its effect on the uterine vasculature during labor. Variables in the exercise protocol that were controlled included the intensity, duration, and type of exercise, the temperature and humidity of the exercise environment, and the diet. We believe that supervision of the training experience is important because pregnant patients are generally unaware of what factors elevate the catecholamine response to exercise and fetal hypoxia is a realistic concern.

A major objective of our conditioning program was to minimize exaggerated postural changes of pregnancy, such as thoracic kyphosis with secondary forward head position and excessive lumbar lordosis. These can occur when muscle groups become shortened or lengthened during gestation, causing a state of imbalance with opposing muscles and producing faulty alignment and deformity. We performed ongoing individual evaluations to identify muscle groups that required strengthening or increase in flexibility in order to compensate for increased mass in abdominal and breast tissue and thereby maintain normal posture.

We believe that pregnant women should not undertake strength-training without supervision because most are unaware of the progressive nature of the postural changes of pregnancy. Unsupervised training may well aggravate lordosis and other symptoms. The rectus abdominis muscle particularly requires strengthening because it is the only longitudinal anterior muscle that controls the degree of lumbar curvature. Failure to strengthen this muscle during pregnancy and to attempt to limit lumbar lordosis and its associated complaints is likely to invite many prenatal aches and pains. More significantly, a previous term pregnancy is one of the most important risk factors for lumbar disc disease.[12] This could be related to failure of the pregnant patient to limit the degree of lordosis during pregnancy and the resulting damage that occurs because of increasing axial compression and/or sheer forces on the lumbar disc.

Goodlin and Buckley[13] have stated that one criterion for judging whether exercise has adverse effects on pregnancy is to compare the size of newborn infants of exercising and nonexercising mothers of similar socioeconomic level. Our data indicated that birth weights were approximately 150 gm higher for babies of women in the high-exercise group than for babies of control mothers (all mothers in our study were middle or upper middle class). In a study of women who followed a running program throughout pregnancy, Clapp and Dickstein[14] found an opposite phenomenon—women who continued exercising to term had offspring whose mean birth weight was 500 gm less than that of offspring whose mothers stopped exercise before the twenty-eighth week of gestation. However, the patients in their report probably consumed more energy during jogging than did the women in our study, whose aerobic exercise consisted of cycling. Oxygen requirements and therefore energy consumption are higher during a weight-bearing exercise such as jogging than during the more mechanically efficient activity of cycling. Thus the attainment of equal degrees of cardiovascular fitness in these two activities requires significantly more caloric expenditure in the case of jogging. In our study, the prescribed length of cycling in controlled environmental conditions should have been minimally energy-draining.

Because of the retrospective nature of this study, no random assignment of subjects to groups was performed, and the numbers of subjects varied from group to group. The most obvious potential problem with retrospective studies of this type is selection bias. Thus undetected intervening variables may have contributed to the superior pregnancy outcomes in our high-exercise group. Possibly the subjects who participated in the conditioning program were more fit to begin with and therefore more predisposed to carry out the training program. However, only four of the 61 women in our high-exercise group—the group clearly demonstrating the best pregnancy outcomes—had a regular exercise history before entering the conditioning program. More controlled experiments are needed to scrutinize the variables that contribute to the impact of physical conditioning on pregnancy outcome.

In no instance did our conditioning program have a negative effect on either the mother or the fetus. This should reassure obstetricians who wish to consider exercise training programs for their pregnant patients. The only minor complaint that some women had was that the treadmill warm-up grew uncomfortable as term approached. For this reason and because of our growing awareness of the need to avoid the effects of gravitational pull on the muscles of the anterior abdominal wall, we discontinued the treadmill warm-up and substituted bicycling. The subjective responses of women in the study to the conditioning program were uniformly positive. They reported that the exercise sessions enhanced their self-image, reduced many minor complaints of pregnancy, and satisfied their need or desire for exercise.

Each pregnant patient has a unique array of modifying factors that makes the individualization of exercise prescription critical. In addition to her physiologic needs, the woman's medical history, vocational and recreational activities, financial and time constraints, and psychological attitudes toward exercise must be considered. Because of the progressive nature of the physical changes of pregnancy, the obstetrician needs to reevaluate the exercise prescription at regular intervals throughout the pregnancy.

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Evaluation of a perinatal grief support team

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An analysis of the effects of intervention from a perinatal grief support team was begun in 1982. Seventy-eight women experiencing stillbirth or early perinatal death were randomly assigned to either the grief support team's protocol of care or to the control group who received routine hospital care. Assessment was carried out on 34 participants, 16 from the control group and 18 from the treatment, 6 months after delivery. A three-part self-administered, self-rating grief index questionnaire was used. There was no statistical difference on overall grief scores between the two matched groups. The treatment group reported significantly lower scores than the control group on the two grief subscales of anger-hostility and physical symptoms. Women who reported more life changes before the perinatal death tended to have fewer problems with the symptomatology of grief. Social support was an important variable in explaining grief symptomatology. Women reporting low levels of social support who were in the treatment group had significantly lower mean scores on the grief subscales of anger-hostility, somatic experience, physical symptoms, and problems with vigor. (AM J OBSTET GYNECOL 1987;157:1203-6.)

Key words: Stillbirth, grief support, perinatal bereavement

The crisis of perinatal death has received attention in the medical literature during the last decade. Health care providers have begun to recognize the need for emotionally supportive care after perinatal death and have implemented programs of active intervention designed to provide that care.

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Evaluation of the efficacy of structured perinatal grief counseling programs is singularly lacking. Forrest et al.[1] provide one evaluation of a group of 50 families who had experienced a perinatal death. Interviews and self-rating scales administered at 6 and 14 months after the death revealed that the unsupported group demonstrated a significantly greater number of psychiatric disorders. Lacking any further documentation of the efficacy of such a program, we undertook an evaluation of a perinatal bereavement intervention program.

A program of active intervention for families experiencing perinatal death was initiated by us at a large tertiary perinatal center serving a predominantly indigent population in west central Florida. As previously

Table I. Objectives of perinatal grief crisis intervention

Before discharge	*4-6 wk post partum*	*4-6 mo post partum*
1. Provide basic human comfort and support	1. Share preliminary autopsy report	1. Share final autopsy results
2. Help make loss real	2. Assess patient's progress	2. Reassess emotional progress
3. Encourage expression of emotion	3. Facilitate emotional release	3. Reassess social support
4. Encourage open communication among family members	4. Describe incongruent grieving	4. Explore possible problems in marital or sexual relationship
5. Prepare for encounters in community	5. Assess couple's progress	5. Schedule anniversary appointment
6. Assess response in preparation for future meetings	6. Discuss local support groups	
7. Record information	7. Assess local support	
8. Schedule 4-6 wk appointment	8. Discuss plans for subsequent pregnancies	
	9. Schedule 4-6 mo appointment	

Table II. Comparison of demographic profiles and obstetric histories of women receiving grief counseling after stillbirth with a control group

	Means for treatment group (N = 18)	*Means for control group (N = 16)*	t *Value*	*Two-tailed value of p from* t *test on means*
Age	25.39	23.93	−0.83	0.411
Education	12.28	11.36	−0.98	0.335
Other babies	1.67	2.62	1.61	0.124
Miscarriages	0.56	0.55	−0.21	0.832
Abortions	0.44	0.07	−1.76	0.092
Age at last pregnancy	26.00	22.43	−2.11	0.046

described, the program focused on a series of timely meetings with the patient, her family, and one or more members of the perinatal grief support team.[2] Two meetings occurred before discharge, another at 4 to 6 weeks after discharge, and a third at 4 to 6 months. Objectives were established for each of those meetings (Table I). A perinatal nurse and perinatal social worker functioned as primary team members.

Methods

Seventy-eight women experiencing perinatal death were enrolled in the study plan between June 1982 and June 1984. Each participant provided informed consent and was then randomly assigned to either treatment group or control group. For the purpose of this study, perinatal death was defined as the delivery of a fetus ≥20 weeks' gestation or weighing at least 500 gm that was either stillborn or died within 2 hours of birth. Intervention was defined as the implementation of the meeting schedule and objectives of the perinatal grief support team. The control group received no intervention from the grief support team.

Both groups were assessed 6 months after delivery by means of a grief index questionnaire. The questionnaire contained (1) questions designed to retrieve demographic, social environment, and health care experience data; (2) the Homes and Rahe social readjustment rating scale,[3] a 39-item inventory that surveyed recent life events; (3) an adaptation of the grief experience inventory.[4] The grief experience inventory, a 63-item inventory that is divided into 16 subscales, asked respondents to agree or disagree with symptoms of grief via a four-point Likert scale.

Participants, after having been contacted by a research assistant who was blinded to the randomization code, were asked to complete the self-administered tool.

Results

Thirty-four of the 78 participants (43.5%) were able to be located approximately 6 months after delivery. Follow-up questionnaires were completed on these 34 patients (16 white, 18 black), 16 from the control group (eight white, eight black) and 18 from the treatment group (eight white, 10 black).

In order to assess comparability of treatment and control groups, *t* tests were run on the means for the demographic variables of age, education, and data on obstetric history (Table II). The treatment and control groups are not significantly different on any variable except age at previous pregnancy. There is also no sampling bias with respect to race nor is race a significant variable in any of the analyses that follow.

Assessment of grief experiences. Treatment and

Table III. Comparison of grief experience scores* of women receiving grief counseling and a control group at 6 weeks after stillbirth

Grief inventory scales	*Mean scores of treatment group (N = 16)*	*Mean scores of control group (N = 18)*	*Two-tailed value of p from t test on means†*
Social desirability	2.58	2.93	NS
Death anxiety	2.33	2.71	NS
Somatic experience	67.60	72.91	NS
Sleep disturbance	8.71	8.85	NS
Appetite	8.00	8.07	NS
Vigor	10.00	10.36	NS
Physical symptoms	22.60	26.16	0.035
Depression	28.79	30.42	NS
Emotional control	10.24	10.06	NS
Anger-hostility	17.41	21.39	0.035
Feelings of unreality	4.82	5.60	NS
Preoccupation with thoughts of deceased	16.20	16.58	NS
Optimism versus despair	3.33	3.27	NS
Guilt	4.39	4.71	NS
Dependency	2.12	2.13	NS
Social isolation	15.22	17.23	0.065
Overall grief score	240.17	259.60	NS

*Higher scores indicate more difficulties.
†*t* test with the use of pooled variance estimate: NS ($p > 0.1$) for all.

Table IV. Analysis of covariance to assess relationship of grief counseling and life changes before death on grief symptoms

	Main effects of treatment		*Effects of life changes before death*		
Grief subscale	*F*	*Significance of F*	*F*	*Significance of F*	R^2
Physical symptoms	10.40	0.004	14.10	0.001	0.527
Anger-hostility	7.97	0.010	8.96	0.007	0.437
Social isolation	5.25	0.032	6.61	0.017	0.350

control group mean scores on the grief experience inventory were compared by means of *t* tests. Comparisons were made on total grief scores as well as subscale scores.

There was no statistically significant difference between the treatment and control groups on overall grief score. The *t* tests comparing mean scores on grief subscales confirmed that a statistically significant difference ($p < 0.05$) exists between the treatment and control groups on the two subscales "physical symptoms" ($p = 0.035$) and "anger-hostility" ($p = 0.035$) (Table III). Patients treated with the perinatal grief counseling protocol experience less difficulty with such physical complaints as diarrhea or constipation, tense neck and shoulders, frequent headaches, dryness of the mouth and throat, and aches and pains as well as with such psychological manifestations of grief as irritability, angry feelings, and resentfulness at the activities of others.

Life stress and grieving. To test the hypothesis that life stress might exacerbate this grief reaction, scores on the Homes and Rahe social readjustment rating scale were calculated for each subject for two periods of time: in the year before and in the time since the death.

To evaluate the relative effects of both grief counseling and life changes on symptomatology of grief, analysis of covariance procedures were used to simultaneously assess the effects of life changes and grief counseling program treatment on those grief subscales that appear to be most significantly related to treatment ("anger-hostility," "physical symptoms," and "social isolation"). When life change in the year before the death is included as a covariate with treatment group status (Table IV), the main effects of treatment remain significant for "physical symptoms" ($F = 10.40$, $p = 0.004$) and "anger-hostility" ($F = 7.97$, $p = 0.010$) and also become significant for "feelings of social isolation" ($F = 5.25$, $p = 0.032$).

Effects of social support on grief and grief counseling. Analysis of questionnaire items designed to assess social support from family, friends, and the father of the baby was carried out. These seven items, taken together, constitute a scale with an alpha reliability co-

efficient of 0.843, a mean score of 19.41, and a range from 8 to 24.

Analysis of covariance procedures to control for the effect of support reveal that, when support is controlled for, treatment effects on "anger-hostility, "physical symptoms," and "feelings of social isolation" become statistically insignificant ($p = 0.29$, 0.18, and 0.09, respectively), suggesting that support is an important variable in explaining grief symptomatology.

Evaluation of the effect of treatment on women reporting low levels of social support, defined as a score below the median of 20, was done. Women with low support scores in both the treatment and control groups were selected out with t tests run on the differences in mean grief scale scores. In women with low support who received counseling, mean scores were significantly lower than for those who were in the control group for "anger-hostility" ($t = 2.04$, $p = 0.059$), "social isolation" ($t = 2.20$, $p = 0.043$), "somatic experience" ($t = 2.59$, $p = 0.022$), "physical symptoms" ($t = 2.07$, $p = 0.059$), and "problems with vigor" ($t = 2.36$, $p = 0.030$).

Comment

In this analysis of the effects of a perinatal grief counseling program on the grief process, several important findings have direct applications for clinicians working in obstetrics. The evaluation has demonstrated that implementation of a grief counseling protocol can reduce aspects of the troubling symptoms of grief after stillbirth with particular effectiveness in certain groups of women. The evaluation has identified women who are most likely to benefit from intervention by a grief support team.

All findings must be evaluated in light of several major methodologic shortcomings. First, all of the questionnaire data were collected 6 months after the deaths of babies and many of the questions demanded retrospective recall by patients. Such an approach is well known to be problematic, as intervening events may selectively enhance or impede recall. Second, our sample is quite small; indeed, this research ought more properly be thought of as a pilot study.

Nonetheless, these data suggest that women who received perinatal grief counseling experienced fewer problems with certain types of grief symptoms. While some of this difference between treatment and control patients may be due to the grief counseling protocol, it is also influenced by other factors.

Our data would suggest, for example, that the amount of life changes a woman has in the year before a stillbirth influences grief symptoms. When we control for life changes and social support, treatment with the protocol predicts fewer problems with anger-hostility and physical symptoms associated with grieving. In those women with low social support, treatment is particularly effective, with women who receive grief counseling reporting less difficulty with feelings of social isolation, anger-hostility, somatic experience, physical symptoms, vigor, and death anxiety than their control subjects who also have low social support.

Clinical implications inferred from this study include the following: (1) Intervention by a grief support team is likely to reduce the grieving woman's anger and hostility; (2) intervention by a grief support team is likely to reduce the physical symptoms characteristic of normal grief; (3) intervention by a grief support team is particularly effective for women who perceive themselves to have low levels of social support. When grieving women receive attention to their emotional needs, particularly women with minimal social support, they are less likely to experience angry feelings and resentfulness regarding the death of their baby. Not uncommonly, health care providers, for a variety of reasons, avoid spending time with grieving patients. This evaluation suggests that that lack of attention may leave women with feelings of anger, hostility, and resentfulness.

Women are frequently troubled by the normal physical symptoms associated with grieving (for example, gastrointestinal distress, insomnia, loss of appetite, headache). Frequent calls and/or visits to the clinician's office are not uncommon as a result. Busy practitioners may in turn be frustrated and angered by a patient they perceive to be a "chronic complainer." This evaluation suggests that intervention by a bereavement team may relieve or decrease the incidence of these troublesome symptoms.

For health professionals working with women who suffer stillbirths, our data would suggest that those women who perceive themselves to have little support from families and friends and who have had very little life change in the year before the deaths of their babies would seem to be at particular risk for troubling grief symptoms and are also those who would most benefit from a special grief counseling program.

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Local peritoneal factors: Their role in infertility associated with endometriosis

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In order to detect peritoneal abnormalities that could account for infertility associated with endometriosis, 122 infertile individuals were studied at the time of laparoscopy for diagnostic purposes or for in vitro fertilization. Four groups were defined: group 1, laparoscopy without endometriosis; group 2, laparoscopy with endometriosis; group 3, in vitro fertilization without endometriosis; and group 4, in vitro fertilization with endometriosis. Mean peritoneal fluid volume was greater, although not significantly so, in group 4 (29.0 ± 6.6 ml, mean ± SEM) than in group 3 (18.2 ± 2 ml). The concentration and total number of pelvic macrophages were similar for groups 1 and 2. The total number of pelvic macrophages was increased in group 4 ($16.9 \pm 4.2 \times 10^6$) versus group 3 ($10.0 \pm 1.8 \times 10^6$) ($p = 0.08$). The mean sperm phagocytosis in vitro did not differ among the four groups studied. Interleukin 1 activity within the peritoneal fluid and the in vitro interleukin 1 production rate did not differ between individuals with and without endometriosis. Peritoneal fluid and macrophage supernatants from individuals with endometriosis were not embryotoxic when studied in an in vitro mouse embryo system. (AM J OBSTET GYNECOL 1987;157:1207-14.)

Key words: Endometriosis, infertility, macrophages, interleukin 1

Although endometriosis is a common finding in a population of infertility patients, there is as of yet no clear cause-and-effect relationship between these two disease entities. When the endometriosis is severe and extensive pelvic adhesions are present, one can implicate a mechanical factor. However, when the endometriosis is mild or moderate and is present without concomitant pelvic adhesions, the mechanism of the infertility associated with endometriosis is not well understood. Indeed it has even been postulated that endometriosis may not cause infertility but may be a result of chronic infertility.

Recently much controversy has been focused on local peritoneal factors and their role in infertility associated with endometriosis. There is some evidence that macrophages may be present in higher concentrations, in higher total numbers, or in a more activated form in patients with endometriosis.[1-3] Peritoneal macrophages from patients with endometriosis have been shown to phagocytize sperm more readily than controls in vitro.[4] Higher prostaglandin concentrations have been demonstrated within peritoneal fluid in individuals with endometriosis by some investigators.[1,5] This derangement of peritoneal fluid chemistry may impair tubal motility or impede the development of the early embryo.

The present investigation deals with pelvic macrophage content and function in infertile patients with and without endometriosis. Sperm phagocytosis was also assessed by an in vitro assay. Interleukin 1 activity in the peritoneal fluid and production rate in vitro were determined. A two-cell mouse embryo system was also used to determine if peritoneal fluid from patients with endometriosis is toxic to the early mammalian embryo.

Material and methods

Subjects. The study population consisted of 122 infertile patients. Seventy-six were studied during diagnostic laparoscopy for infertility: Group 1 (n = 41) was without endometriosis whereas group 2 (n = 35) displayed active endometriosis. Forty-six patients were studied during laparoscopy for egg retrieval and in vitro fertilization: Group 3 (n = 32) was without endometriosis whereas group 4 (n = 14) displayed active endometriosis. All patients had undergone a complete infertility investigation including analysis of semen, basal body temperature graphing, timed progesterone determination, hysterosalpingography, and postcoital testing. Patients with a history of endometriosis but no active disease at the time of laparoscopy were excluded from the study.

Sample collection. Laparoscopy was performed by one of the authors with the patient under general anesthesia. A careful search for endometriosis was made

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by use of a double-puncture technique. Endometriosis was staged according to the original American Fertility Society classification. Peritoneal fluid was aspirated from the anterior and posterior cul-de-sacs with a hollow cannula. This was accomplished before placement of the patient in the Trendelenburg position. The peritoneal fluid was then placed into sterile heparinized tubes in order to avoid macrophage adherence to glass. The fluid was immediately transported to the laboratory. Its volume was determined to the nearest 0.5 ml and the fluid was centrifuged at 500 × g for 10 minutes. The supernatant was then removed and used for analysis on the same day or frozen at −20° C until later assay. The cell pellet was then resuspended in 4 ml of phosphate-buffered saline solution and recentrifuged at 500 × g for 10 minutes. The supernatant was removed and the cells resuspended in 1 ml of Ham's F-10 medium (Gibco, Grand Island, New York) with 10% fetal calf serum (Sigma, St. Louis, Missouri) supplemented with 20 μg/ml penicillin and 12.5 μg/ml streptomycin. Twenty microliters of the suspension was then diluted 1:10 with phosphate-buffered saline solution and stained with 1% trypan blue. With a hemacytometer, five large (1 mm) squares were counted and the concentration of viable leukocytes was determined. A cytospin preparation of the cell suspension was made, fixed in alcohol, and stained with Giemsa. A differential count was then performed. Macrophages can be recognized because of their large size and vacuolization.

Sperm phagocytosis. Sperm was obtained from fertile donors who were participants in an artificial insemination program. Fresh or cryopreserved sperm was used. Cryopreserved sperm displayed a motility of 40% to 60%. Fresh sperm typically displayed 60% to 80% motility. Sperm was doubly washed with Ham's F-10 medium with 10% fetal calf serum and the final suspension was adjusted to a concentration of 1×10^7 sperm per milliliter. The macrophage suspension was adjusted to a concentration of 4×10^6 macrophages per milliliter. One tenth milliliter of the macrophage suspension was placed within a microtiter chamber. The macrophages were allowed to attach for 2 hours. Each well was then washed with Ham's F-10 medium in order to remove nonadherent cells. Nonadherent cells consisted primarily of lymphocytes, erythrocytes, and nonviable macrophages. The number of viable macrophages within the supernatant was consistently ≤10% of the originally plated number. The number of adherent cells per well did not differ among the groups studied. One hundred microliters of sperm was added to each of three chambers with macrophages and to each of three empty chambers to be used as controls. The samples were incubated for 20 hours. The supernatant was then harvested by pipetting the fluid up and down five times. The sperm were counted in a hemacytometer. The percent phagocytosis is expressed as:

$$100 - \frac{\text{number of sperm in supernatant of experimental well}}{\text{number of sperm in control supernatant}} \times 100$$

The intra-assay variability was 7%.

Interleukin 1 assay. Interleukin 1 activity within the peritoneal fluid and the in vitro production rate of interleukin 1 were determined. When peritoneal fluid levels were determined, the peritoneal fluid was stored at −20° C until the day of the assay. When interleukin 1 levels in culture media were determined, 1×10^5 macrophages were cultured in a microtiter well with 0.2 ml of Ham's F-10 medium and 10% fetal calf serum. The culture medium was harvested at 20 hours and frozen at −20° C until assay. In a separate experiment, macrophage production of interleukin 1 was stimulated with 20 μg/ml of lipopolysaccharide.

Interleukin 1 assay was performed as described by Lachman et al.[6] Male CD-1 mice, 4 to 8 weeks old (Charles River, Wilmington, Massachusetts), were put to death and the thymuses were removed and pressed through a wire mesh aseptically. The debris was allowed to sediment and the thymocytes were washed and resuspended to a concentration of 1×10^7/ml in medium RPMI 1640 plus 100 μ/ml penicillin, 100 μ/ml streptomycin, 0.3 mg/ml glutamine, 2.5×10^{-5} mol/L 2-mercaptoethanol, and 5% AB serum. Cultures were established in microtiter plates with each well containing 1×10^6 cells. Each experiment was performed in triplicate. Ten microliters of culture medium to be tested or peritoneal fluid was added to each well. Each experiment was controlled. Controls consisted of Ham's F-10 medium with 10% fetal calf serum. Cultures were incubated at 37° C with 5% carbon dioxide and pulsed after 48 hours with 0.5 μCi tritium-labeled thymidine (New England Nuclear, Boston, Massachusetts). Cultures were harvested at 72 hours onto filter paper with an automatic harvester. The incorporated radioactivity was measured by means of a liquid scintillation counter. Interleukin 1 activity was expressed as average counts per minute.

Mouse embryo assay. Two-cell mouse embryos were used as the starting point of the assay. CB_6F_1 mice, both male and female (Charles River), were used. This is a hybrid cross between Balb/c and c57B1/6 strains and have provided two-cell embryos whose rate of development to blastocyst is ≥70%. Ovulation was induced with 7.5 IU of pregnant mare serum intraperitoneally on the first day of estrus. This was followed by 7.5 IU human chorionic gonadotropin intraperitoneally 48 hours later. On the day of human chorionic gonado-

Table I. Characteristics of the study groups

Group	n	Age (yr) (mean ± SEM)	Duration of infertility (yr) (mean ± SEM)	% with primary infertility
1	41	29.7 ± 1.1	3.0 ± 0.4	46
2	35	30.7 ± 1.2	3.5 ± 0.5	74
3	32	31.2 ± 1.0	5.0 ± 0.5	44
4	14	32.7 ± 1.1	5.2 ± 0.5	86

Table II. Total content and concentration of pelvic macrophages

Group	n	Concentration of macrophages × 10^5/ml (mean ± SEM)	Total macrophages × 10^6/case (mean ± SEM)
1	41	11.07 ± 1.3	8.16 ± 1.1
2	35	12.9 ± 1.8	9.79 ± 1.3
3	32	6.06 ± 0.65	10.0 ± 1.8
4	14	6.61 ± 1.4	16.9 ± 4.2

tropin administration the female mice were mated with male mice of established fertility in a 1:1 ratio. Forty-eight hours later the female mice were put to death by cervical dislocation and the fallopian tubes were dissected. Under the dissecting microscope, the fallopian tubes were incised and two-cell mouse embryos were pipetted into one dish containing Ham's F-10 medium. All embryos were pooled before random allocation to experimental wells. During each trial, equal numbers of endometriosis and nonendometriosis specimens were analyzed. Experiments in which the control result was less than 70% blastocyst formation were repeated. Control wells consisted of Ham's F-10 medium with 10% fetal calf serum. For testing of peritoneal fluid, wells with 10% peritoneal fluid were prepared. For testing macrophage supernatants, 0.5×10^5 macrophages were placed in a microtiter well and allowed to adhere for 2 hours; then the plates were washed with Ham's F-10 medium. One hundred microliters of Ham's F-10 medium with 10% fetal calf serum or Ham's F-10 medium with 10% fetal calf serum containing 10^{-5} mol/L indomethacin or 20 μg/ml lipopolysaccharide was then added to the adherent macrophages. After 24 hours 100 μl of the supernatant was transferred to a new well and to this was added 100 μl of fresh Ham's F-10 medium with 10% fetal calf serum. To each well eight to 10 two-cell mouse embryos were added. A layer of paraffin oil was used to cover the wells and the embryos were incubated for 72 hours. The numbers of blastocysts and degenerate embryos were then determined under phase contrast microscopy.

Statistics. Statistical analysis was conducted by use of the unpaired *t* test and the Mann-Whitney U test. Analysis of variance was conducted to evaluate the mouse embryo experiment and the sperm phagocytosis experiment.

Results

Seventy-six patients were studied during diagnostic laparoscopy for infertility. Within group 2, 77% of patients displayed mild endometrosis and the remainder displayed moderate endometriosis. Seventy-three percent of patients in group 1 displayed pelvic adhesions whereas the remainder had normal pelvic anatomy and their infertility could be explained on the basis of ovulatory dysfunction, cervical factor, or male factor. Forty-six patients were studied during laparoscopy for egg retrieval and in vitro fertilization. Within group 4, 79% displayed mild endometriosis and the remainder displayed moderate endometriosis. Seventy-four percent of patients in group 3 underwent the procedure because of tubal disease; in the remainder, the indication was male factor or cervical factor infertility. Table I details general characteristics of the four study groups.

Peritoneal fluid volume was found to be increased in the luteal phase as compared with the follicular phase in both and groups 1 and 2, as shown in Fig. 1. The mean peritoneal fluid volume was 6.78 ± 0.92 ml (±SEM) for group 1 individuals in the follicular phase (n = 18) compared with 12.9 ± 3 ml for group 1 individuals (n = 19) in the luteal phase (p = 0.05). The mean peritoneal fluid volumes in the follicular (n = 19) and luteal phases (n = 13) for group 2 were 8.09 ± 1 and 17.2 ± 5.3 ml, respectively (p = 0.04). The overall mean peritoneal fluid volume for group 2 was 9.36 ± 0.99 versus 9.59 ± 1.5 for group 1 (NS). No statistically significant difference in peritoneal fluid volume was found between groups 1 and 2 when analysis was restricted to either the follicular or the luteal phase of the menstrual cycle. When group 2 was analyzed on the basis of the stage of endometriosis, the peritoneal fluid volume was 9.44 ± 1.2 ml for 27 patients with mild endometriosis and 9.09 ± 1.2 ml for eight patients with moderate endometriosis (NS); neither value was significantly different from the value in group 1 (9.59 ± 1.5 ml, n = 41). The mean peritoneal fluid volume for group 4 patients was higher than for group 3 patients (29.0 ± 6.6 versus 18.2 ± 2 ml). However, this difference was not statistically significant (p = 0.10).

Table II displays data for the concentration and total numbers of pelvic macrophages. No statistically significant differences were found when the concentration or total number of pelvic macrophages was compared between groups 1 and 2. When patients with mild endometriosis were compared with those with moderate

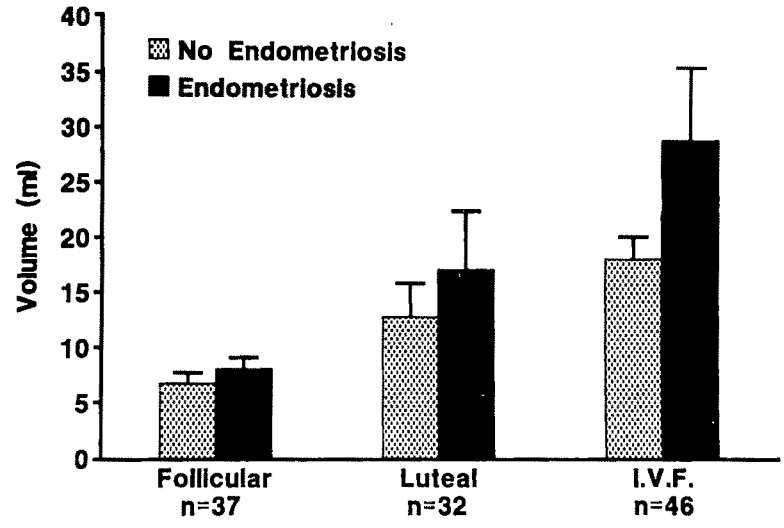


Fig. 1. Peritoneal fluid volume (mean ± SEM) during various phases of the menstrual cycle.

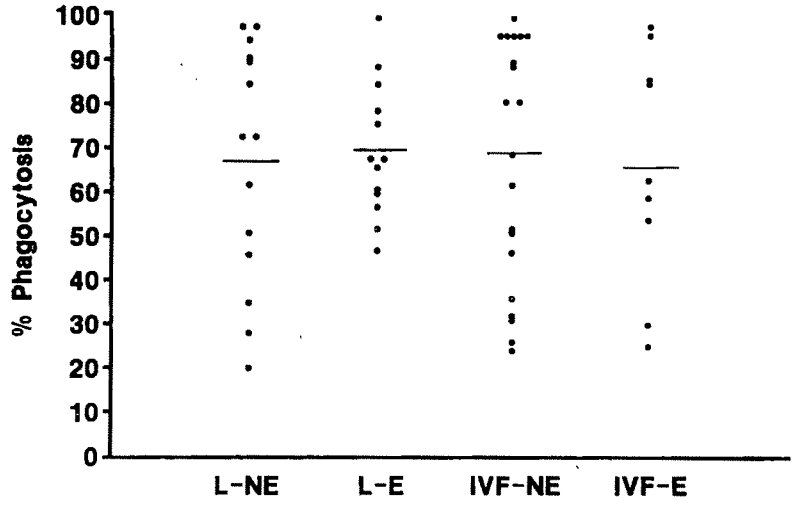


Fig. 2. In vitro sperm phagocytosis in patients undergoing laparoscopy with no endometriosis (group 1, *L-NE*), laparoscopy with endometriosis (group 2, *L-E*), in vitro fertilization with no endometriosis (group 3, *IVF-NE*), and in vitro fertilization with endometriosis (group 4, *IVF-E*).

endometriosis within group 2, no significant differences were discovered in the mean concentration or total number of pelvic macrophages. For groups 3 and 4, no difference was found in the concentration of pelvic macrophages. The total content of macrophages was 16.9 ± 4.2 × 10^6 (± SEM) in group 4 patients and 10.0 ± 1.8 × 10^6 in group 3 patients (p = 0.08).

The results of the sperm phagocytosis assay for the four groups studied are shown in Fig. 2. The mean phagocytosis was 69.8% ± 4.3% for group 2 patients (n = 13) versus 67.5% ± 7.3% for group 1 patients (n = 14) (NS). The mean sperm phagocytosis was 66.2% ± 9% for group 4 patients (n = 9) versus 69.2% ± 6.3% for group 3 patients (n = 20) (NS). The mean sperm phagocytosis was 68% ± 5.4% for patients with mild endometriosis (n = 16) compared with 69.3% ± 7.4% for individuals with moderate endometriosis (n = 6) (NS); neither of these values differed significantly from those of group 3. Very little nonspecific adherence of sperm to plastic was noted. Initial and final counts in control wells varied by less than 5%. Since the assay measures phagocytosis and adherence of sperm to macrophages, phase contrast microscopy after incubation and removal of supernatant for counting was performed. Little adherence of sperm was noted, suggesting that the majority of assayable activity is phagocytosis rather than adherence. Sperm phagocytosis was relatively rapid with most of the phagocy-

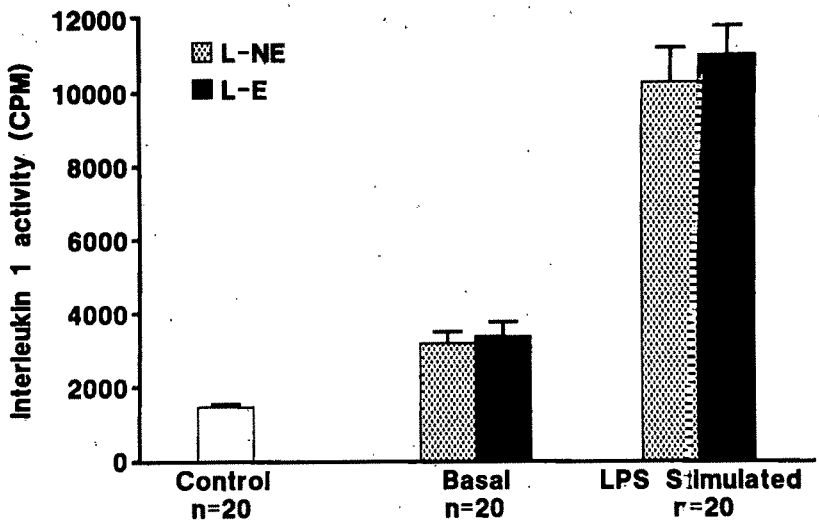


Fig. 3. In vitro production rate of interleukin 1 expressed as mean ± SEM for the group 1 *(L-NE)* and group 2 *(L-E)*. *LPS* = Lipopolysaccharide.

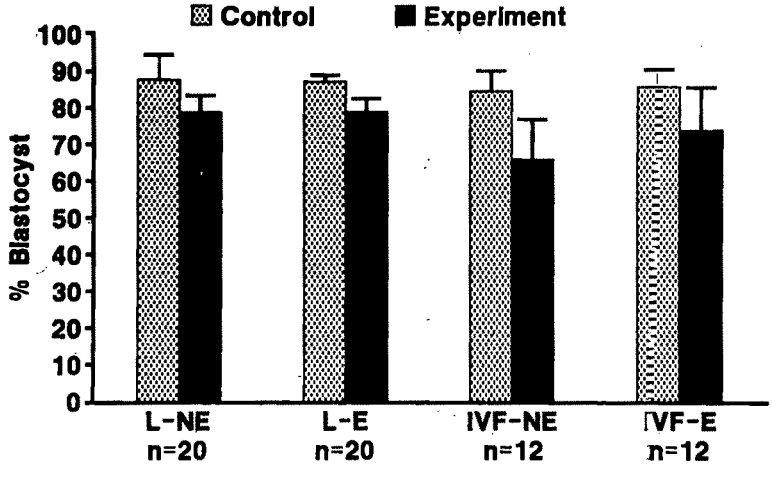


Fig. 4. Effect of peritoneal fluid on mouse embryo development in vitro. Results expressed as mean ± SEM for the following groups: laparoscopy with no endometriosis (group 1, *L-NE*); laparoscopy with endometriosis (group 2, *L-E*), in vitro fertilization with no endometriosis (group 3, *IVF-NE*), and in vitro fertilization with endometriosis (group 4, *IVF-E*).

tosis completed by 6 hours. At 6 hours of incubation 85% ± 5.6% of the phagocytic activity observed at 20 hours had occurred (n = 10).

Peritoneal fluid interleukin 1 levels were determined in a single assay for 14 patients in group 1 and 14 patients in group 2. Peritoneal fluid interleukin 1 activity was 3233 ± 468 and 3331 ± 600 cpm for groups 1 and 2, respectively (NS). Fig. 3 displays the interleukin 1 activity in macrophage supernatants after 20 hours of in vitro culture. No difference was found in the in vitro production rate of interleukin 1 by macrophages from groups 1 and 2 under basal or lipopolysaccharide-stimulated conditions. Also there did not appear to be a statistically significant difference in peritoneal fluid levels or production rates of interleukin 1, depending on the proliferative versus the secretory phase of the menstrual cycle.

The mouse embryo experiment was conducted with peritoneal fluid in 64 cases. Fig. 4 displays the results. Since each experiment was controlled, each result is listed with its own control consisting of Ham's F-10 medium with 10% fetal calf serum. The mean percent of two cells developing to the blastocyst stage was 79.2 ± 4.4 for group 1 versus 79.3 ± 3.5 for group 2 and 87.7 ± 1.61 for the control group. This difference was not statistically significant. The mean percent of two cells developing to the blastocyst stage was 66.3 ± 10.8 for group 3 versus 73.8 ± 11.7 for

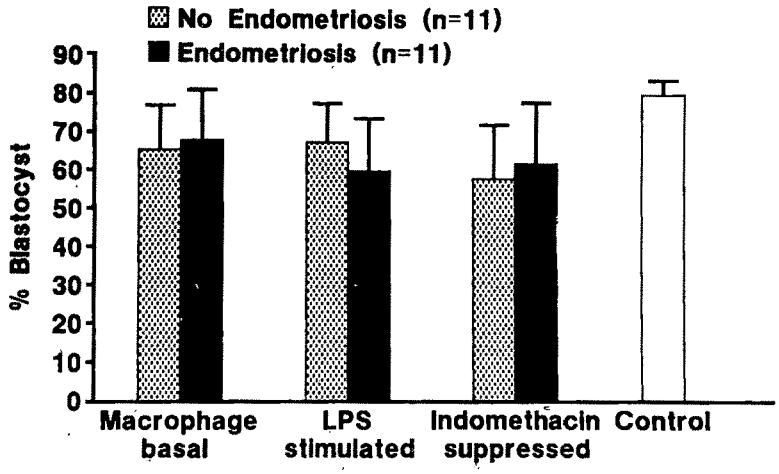


Fig. 5. Effect of macrophage supernatants on mouse embryo development in vitro, expressed as mean ± SEM.

group 4 and 85 ± 4.59 for the control group. This difference also was not statistically significant.

Culturing of mouse embryos in macrophage supernatants was accomplished in 11 patients with and 11 patients without endometriosis. Fig. 5 displays the results of these experiments. No statistically significant differences in the rate of development to the blastocyst stage were found when each of the experimental groups was compared with the control group. Also no statistically significant differences between any of the experimental groups were found.

Comment

Although endometriosis is a frequent finding in the setting of infertility, the pathophysiologic characteristics of infertility in the setting of endometriosis are not well defined. Recently much attention has focused on local peritoneal factors and their role in infertility associated with endometriosis. Analysis of peritoneal fluid volumes is hampered by variance of the volume as a function of the menstrual cycle. Also it is important to differentiate whether patients with endometriosis are compared with normal fertile individuals or with other infertile individuals without endometriosis. Oak et al.[7] and Drake et al.[8] have noted an increased volume of peritoneal fluid in patients with endometriosis when compared with fertile control subjects. Numerous other investigators have reported no difference in peritoneal fluid volume in individuals with endometriosis when compared with other infertile patients.[9, 10] Peritoneal fluid volumes within the context of this investigation were not found to be significantly different when infertile patients with and without endometriosis were compared at the time of diagnostic laparoscopy. At the time of in vitro fertilization, the mean peritoneal fluid volume was higher in patients with endometriosis but this difference was not statistically significant ($p = 0.10$).

Recently much attention has centered on pelvic macrophages as a possible mediator of infertility in patients with endometriosis. Macrophages have been reported to consistently represent at least 90% of peritoneal leukocytes.[4] Macrophages have also been demonstrated within the lumen of the fallopian tube. Therefore they have access to the microenvironment of fertilization and early embryonic development. Verifying differences in macrophage numbers or function would be an important step in implicating macrophage dysfunction as causative in infertility associated with endometriosis. Within the four groups studied no significant differences were found in macrophage concentrations or total pelvic content. The mean total pelvic macrophage content was higher for group 4 than group 3 (16.9 ± 4.2 versus 10.0 ± 1.8) but this difference was not significant. It is to be emphasized that our control group consisted of infertile patients without endometriosis. Badawy et al.[1] demonstrated increased numbers of peritoneal macrophages in patients with endometriosis when compared with infertile control subjects ($n = 102$). Haney et al.[2] demonstrated increased macrophage counts in women with endometriosis when compared with normal fertile control subjects or other infertile individuals with endometriosis ($n = 32$). Halme et al.,[3] in 1983, reported no statistically significant differences in pelvic macrophage concentration when comparing infertile women with endometriosis and either fertile or infertile control subjects ($n = 66$). However, they did report evidence of increased macrophage activation in patients with endometriosis. In 1984, Halme et al.[11] again reported the finding of similar or lower concentrations of pelvic macrophages in individuals with endometriosis when compared with

fertile control subjects (n = 107). From this conflicting information one might suggest that the populations studied are probably not homogeneous. Perhaps some patients with endometriosis do possess an increased total number of macrophages since a trend toward this was found in this investigation.

Macrophages originate in the bone marrow and circulate in the blood in the form of monocytes. They migrate to various sites of the body, differentiate into macrophages, and serve as an immunologic line of defense for the human body. Macrophages in particular are highly phagocytic and normally remove foreign particles and cellular degradation products from the body. Muscato et al.[4] demonstrated in 1982 that macrophages from infertile patients with endometriosis phagocytized more sperm than macrophages from infertile patients without endometriosis. The sample size was 32 individuals including 10 patients who had endometriosis. To our knowledge, this result has not been duplicated. However, the phagocytosis assay was different from the current investigation in that the concentration of macrophages and the total number of sperm used were different. The hypothesis that increased phagocytosis of sperm may account for infertility associated with endometriosis is an attractive one. This is particularly so since there is evidence that macrophages are present within the fallopian tubes and it is hypothesized that these may be of peritoneal origin.[12] However, Stone and Himsl[13] recently demonstrated in laparoscopies performed 2-4 hours after insemination that sperm recovery from the cul-de-sac was not significantly different between infertile patients with and without endometriosis. Since neither an increased concentration of macrophages nor increased sperm phagocytosis by existing macrophages was encountered in this investigation, no evidence was found to support the hypothesis that increased sperm phagocytosis is causative in infertility associated with endometriosis.

Macrophages are highly metabolic cells. Their secretory products include prostaglandins, thromboxanes, hydrolytic enzymes, and lymphokines. Recently much interest has focused on peritoneal fluid concentrations of prostaglandins and leukotrienes in infertile patients with endometriosis. These compounds may alter tubal motility and thus impair fertility. Drake et al.[5] demonstrated increased levels of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ in patients with endometriosis. DeLeon et al.[10] demonstrated higher concentrations of prostaglandin $F_{2\alpha}$, prostaglandin E_2, thromboxane B_2, and 6-keto-prostaglandin $F_{1\alpha}$ in patients with endometriosis. However, the data are not completely clear. Rock et al.,[9] restricting their analysis to the late proliferative phase, found no difference in concentrations of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and thromboxane B_2 in patients with and without endometriosis. In this investigation, a new secretory product of macrophages, interleukin 1, was measured. Macrophages are involved in two ways in antigen-specific T-cell recognition. They must bind and present antigens to the T cells. Also macrophages produce a peptide known as interleukin 1 with a molecular weight of approximately 15,000 daltons. Interleukin 1 induces T-cell production and release of interleukin 2, which causes proliferation of T cells.[14] To our knowledge, no previous study has quantitated peritoneal fluid levels of interleukin 1 or peritoneal macrophage production rates of interleukin 1 in patients with and without endometriosis. In this investigation, no differences in these two parameters were found when patients with and without endometriosis were compared. Recently, Cannon and Dinarello[15] reported increased serum levels of interleukin 1 in the luteal phase. It is of interest that no variation of interleukin 1 activity within peritoneal fluid was found during the menstrual cycle. However, it should be noted that our samples were cross sectional and perhaps obscured individual cycle variations.

Because of the various aberrations in peritoneal fluid that have been reported, there is concern that peritoneal fluid from patients with endometriosis may be toxic to gametes or early embryos. Morcos et al.,[16] using a two-cell mouse embryo system, reported a significant lowering of the rate of development to the blastocyst stage when mouse embryos were incubated in a solution of 5% peritoneal fluid (heat inactivated). However, the sample size was small (n = 28). In the current investigation with a sample of 64 peritoneal fluid specimens, we could not demonstrate a difference in the rate of development to the blastocyst stage in patients with and without endometriosis. Also no difference was found between the peritoneal fluid groups and the control group. Of note is the fact that we did not heat inactivate the peritoneal fluid. To document further whether macrophages produce substances that are embryotoxic, the growth of two-cell mouse embryos was studied in solutions containing supernatant from macrophage cultures. Since lipopolysaccharide is known to stimulate macrophage production of interleukin 1 and prostaglandins, macrophages were also stimulated with lipopolysaccharide and suppressed with indomethacin. No statistically significant differences either between experimental groups or between each group and the control group were demonstrated. These results suggest then, in the early mouse embryo model, that peritoneal fluid from patients with endometriosis is nontoxic. Also peritoneal macrophages do not appear to produce in vitro metabolites or products that are toxic to the early mouse embryo.

In this investigation, with a sample size of 122 individuals, a significant difference in peritoneal fluid volume, in concentration of macrophages, or in total con-

tent of pelvic macrophages could not be demonstrated. No significant difference in macrophage-mediated sperm phagocytosis in vitro was found. Interleukin 1 levels in peritoneal fluid and the production rate in vitro were similar for patients with and without endometriosis. Peritoneal fluid from patients with endometriosis did not appear to impede the two-cell mouse embryo from development to blastocyst. Peritoneal macrophages from patients with endometriosis did not appear to produce embryotoxic substances.

The search for an etiologic connection between endometriosis and infertility has been a complex one. Conflicting data appear to be developing more rapidly than consensus information. Small sample size is a significant problem in this literature. Also cycle analysis is quite significant in some parameters, for example, peritoneal fluid volume. The selection of adequate control groups is also problematic; some investigators use infertile patients without endometriosis as controls while others choose fertile individuals who are to undergo sterilization. Bioassays are also typically quite variable and require painstaking controls in order to yield meaningful results. Also individuals with endometriosis may not form a homogeneous population. This may be due at least in part to the extent of the disease. Within the context of this investigation, we could not definitively demonstrate an abnormality in the local peritoneal environment that could account for the infertile state associated with endometriosis.

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The morphologic effect of short-term medical therapy of endometriosis

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A clinicopathologic study of a short-term medical treatment of endometriosis with danazol or gestrinone was performed in 50 patients with endometriosis and infertility. The cellular response to the therapy was evaluated and graded according to morphologic, that is, histologic and ultrastructural, criteria. A 2-month therapy with 1.25 mg of gestrinone daily induced a degree of cellular inactivation and degeneration of the endometriotic implants that was more pronounced than after 4 months of therapy with either gestrinone (2.5 mg twice or thrice weekly) or danazol (600 mg daily). There was no correlation between the morphologic response to treatment and either the hormonal response of the foci during the menstrual cycle before therapy or the change in laparoscopic staging of endometriosis at the end of therapy. It is suggested that a short-term medical treatment should be further evaluated clinically in the treatment of endometriosis and infertility. (Am J Obstet Gynecol 1987;157:1215-21.)

Key words: Endometriosis, danazol, gestrinone

Clinical studies of the treatment of endometriosis in infertility are faced with major difficulties in evaluating the effects of treatment. The ultimate parameter in infertility is pregnancy. However, the relationship between endometriosis and infertility is complex and many unrelated factors may interfere with the outcome. Large, prospective and controlled clinical studies are difficult to perform. Laparoscopy has been used to evaluate the effect of medical treatment and a variable degree of resolution has been described after long-term therapy.[1] Recent ultrastructural studies have detailed the cellular changes in the endometriotic implants during suppressive hormonal therapy with either danazol[2, 3] or gestrinone.[4, 5] Long-term therapy with danazol results in cellular inactivity and a variable degree of cellular disintegration but not in disappearance of the implant[2] and is associated with multiple side effects and metabolic changes. In view of these considerations a clinicopathologic study was performed to compare the morphologic effects of a 4-month versus a 2-month regimen of medical therapy of endometriosis in patients with infertility.

Material and methods

Clinical data. Fifty patients with endometriosis and infertility were admitted to the study. The clinical diagnosis of endometriosis was confirmed by laparoscopy in all patients and one or more biopsies were performed in suspected foci endometriosis. After the clinical diagnosis the patients were treated with either danazol or gestrinone for periods of 4 or 2 months (Table I). At the end of therapy laparoscopy was performed and the degree of endometriosis was assessed according to the revised American Fertility Society classification system.[6] Small biopsy specimens were taken from the suspected endometriotic foci with a 3 mm punch biopsy forceps (Wolf Endoskope, Knittlingen, West Germany). Ovarian and/or peritoneal biopsies were performed in 18 patients before and after therapy, in seven patients before therapy only, and in 25 patients after therapy only.

Table I. Schemes of medical therapy and number of patients

Product	*Dose*	*Duration of therapy (months)*	*No. of patients*
Danazol	600 mg daily	4	12
Gestrinone	2.5 mg twice or thrice weekly	4	20
	1.25 mg daily	2	18

Morphologic techniques. Tissues were immediately immersed in cold (4° C) 0.1 mol/L phosphate-buffered (pH 7.2) 1.25% glutaraldehyde. After this primary immersion they were cut into small blocks, their size not exceeding 2 mm^3. In total, 360 tissue blocks were sampled (74 tissue blocks of material obtained before treatment and 286 tissue blocks after treatment) and further processed for histologic and transmission electron microscopic examination as described previously.[4] Semi-

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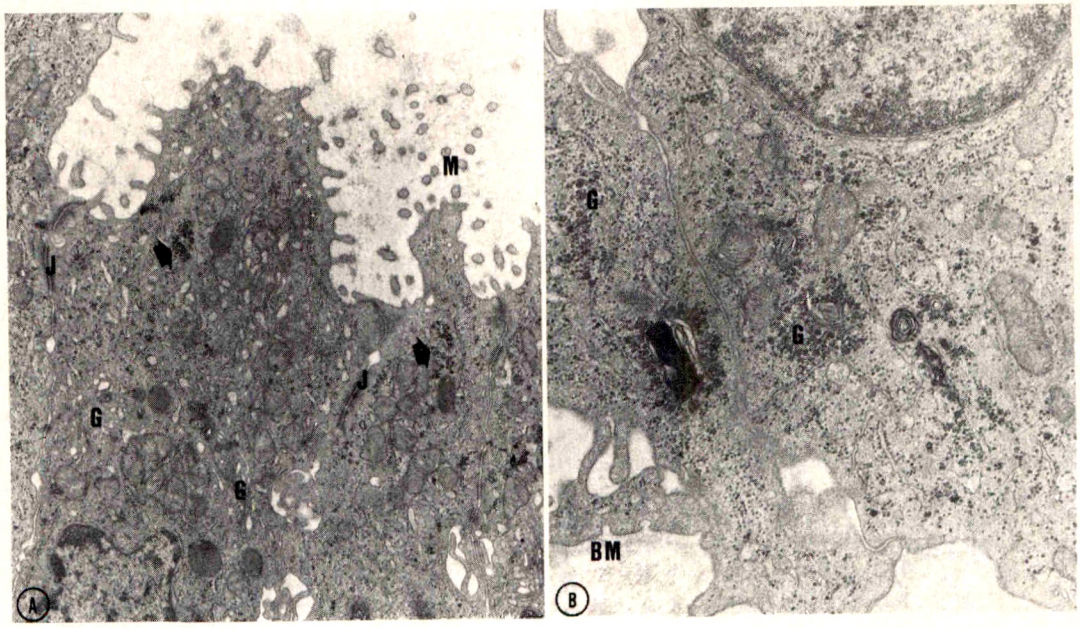


Fig. 1. *A*, No therapy response. Well-differentiated apical cell regions of endometriotic epithelial cells from the uterosacral ligament after a 4-month gestrinone therapy (2.5 mg thrice weekly). Note the well-developed Golgi complexes *(G)*, the small patches of glycogen granules *(arrows)*, the apical epithelial junctions *(J)*, and the numerous microvilli *(M)*. (Original magnification ×14,000.) *B*, Basal epithelial cell regions of the same implant shown in *A*. Small aggregations of glycogen particles *(G)* are present throughout the subnuclear cytoplasm. *BM* = Basement membrane. (Original magnification ×28,500.)

Table II. Morphologic response during menstrual cycle and site of biopsy

Site of endometriosis	*Total No. of biopsy specimens*	*Positive biopsy specimens*		*Hormonal response during cycle*					
				0		*1*		*2*	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Ovary	10	7	70	3	43	3	43	1	14
Peritoneum	21	19	90	8	42	6	32	5	26
Total	31	26	84	11	42	9	35	6	23

thin (1 μm) sections were cut with a Sorvall Porter Blum MT2 ultramicrotome (Du Pont Company, New York, New York) by use of glass knives. From each tissue block several semithin sections were mounted and stained with toluidine blue according to the method of Humphrey and Pittman.[7] They were screened for the presence of endometriotic lesions including endometrial surface and/or glandular epithelium to confirm a histologic diagnosis of endometriosis.

From 39 patients 95 tissue blocks bearing endometriotic foci were studied by transmission electron microscopy. Thin sections (50 to 70 nm) were cut with an LKB ultramicrotome (LKB Instruments, Sweden) by use of a diamond knife. They were stained with uranyl acetate and lead citrate[8] and examined with a Zeiss 9 electron microscope (Zeiss).

The morphologic changes of the endometriotic foci during the menstrual cycle were graded as follows: Grade 0 = undifferentiated endometriotic epithelium and stroma without proliferative and/or secretory changes (no response); grade 1 = weak response with only scanty proliferative and/or secretory transformation within part of the epithelium and stroma; grade 2 = good response with clear proliferation and/or secretion in many endometriotic cells. This morphologic staging according to the differentiation of the endometriotic foci was done for all pretreatment biopsy specimens that were not timed with respect to ovula-



Fig. 2. Weak therapy response. These endometriotic epithelial cells from the uterosacral ligament reveal an undifferentiated aspect lacking proliferative and secretory transformations. Biopsy performed after 4 months of gestrinone therapy (2.5 mg twice weekly). (Original magnification ×5400.)

tion. Thus, the aim of this staging was to evaluate the endocrine dependency of the implants during the cycle. It was shown earlier that endometriotic implants usually reveal less cellular differentiation than eutopic endometrium, while some implants may show no differentiation at all.[9] Furthermore, differentiation of endometriotic foci may be out of phase with that found in the endometrium. According to Schweppe and Wynn,[9] this staging of the differentiation of endometriotic implants should correlate with the degree of therapeutic response, that is, well-differentiated foci (type 2 hormonal response during the cycle) should reveal good response to medical therapy (grade 2 morphologic changes; see below).

The morphologic changes during therapy as described by Cornillie et al.[4] were graded as follows: grade 0 = no response with focal proliferative and/or secretory changes still present (Fig. 1, *A* and *B*); grade 1 = weak response; the endometriotic foci are undifferentiated but lacking clear involutionary changes (Fig. 2); grade 2 = good response with marked cellular involution and degradation (Fig. 3, *A* and *B*). All biopsy specimens were evaluated by one of the authors (F. C.) in the absence of knowledge of clinical data.

Results

Before therapy. The diagnosis of endometriosis was confirmed morphologically in 84% of the biopsies performed before treatment. The scoring of the hormonal response during the menstrual cycle is given in Table II. The hormonal response was absent in 42% of all biopsy specimens, weak in 35%, and good in 23%.

Therapeutic response. The diagnosis of endometriosis was confirmed in 68% and 78% of the biopsy specimens taken after 4 and 2 months of therapy, respectively (Tables III and IV). The scoring of the therapeutic response according to the site of biopsy is given in Tables III and IV. During both regimens of therapy good response was seen more frequently in peritoneal

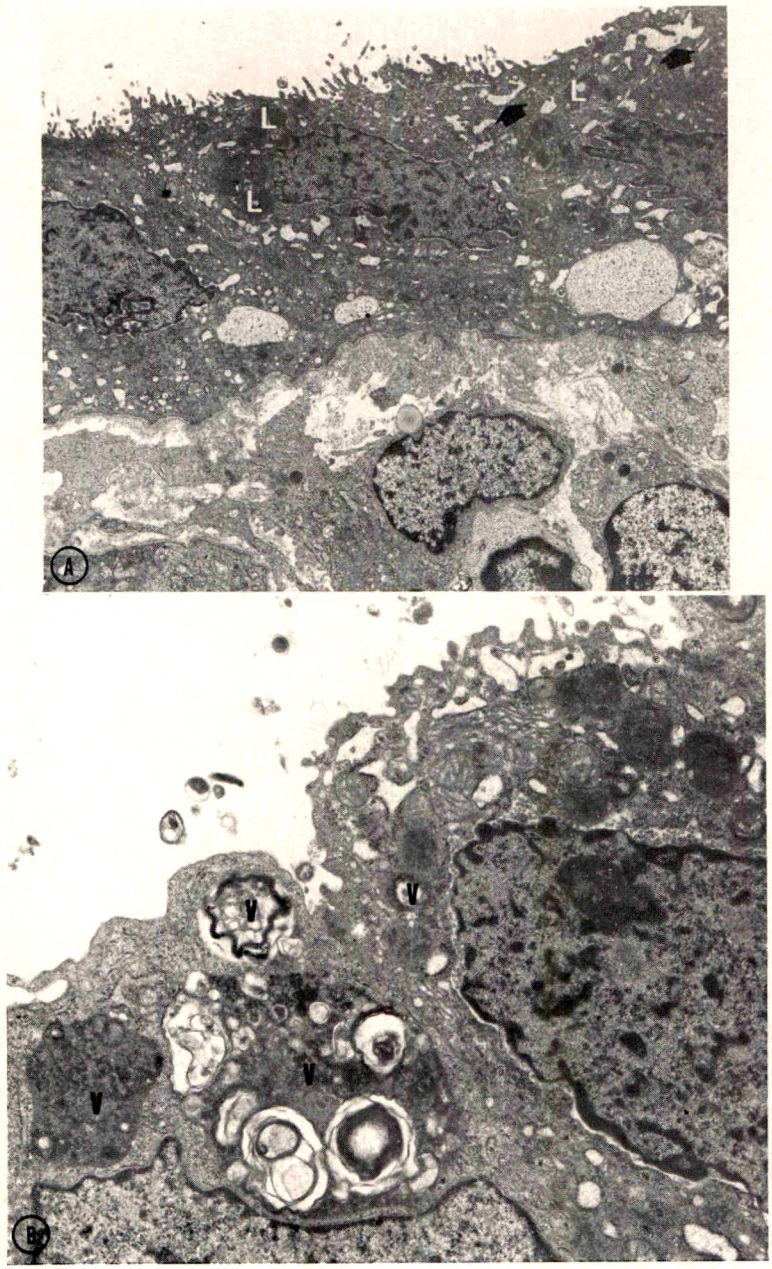


Fig. 3. *A*, Good therapy response. Low-power survey of the epithelial changes after a 2-month therapy with gestrinone, 1.25 mg daily. Note the irregular nuclear outline, the dilatation of rough endoplasmic reticulum cisternae *(arrows)*, and the numerous electron-dense lysosomes *(L)* within the apical cytoplasm. Implant from the uterosacral ligament. (Original magnification ×5400.) *B*, Larger detail of ultrastructural changes seen in endometriotic epithelial cells with good therapy response. Large autophagic vacuoles *(V)* are present within the supranuclear cytoplasm. Same implant as shown in *A*. (Original magnification ×28,500.)

than in ovarian biopsy specimens. There was a lack of response during the 4-month therapy in both peritoneal and ovarian biopsy specimens and during the 2-month therapy in ovarian biopsy specimens only. Also the therapeutic response to gestrinone therapy was more pronounced in peritoneal biopsy specimens after a 2-month therapy with 1.25 mg daily than after a 4-month therapy with either 600 mg of danazol daily or 2.5 mg of gestrinone twice or thrice weekly (Table V). No significant difference in the grading of morphologic response was found between the 4-month treatment with 2.5 mg of gestrinone twice or thrice weekly and 600 mg of danazol daily.

No correlation could be demonstrated between the

Table III. Response to therapy and site of biopsy after a 4-month therapy

Site of endometriosis	Total No. of biopsy specimens	Positive biopsy specimens		Morphologic response to therapy					
				0		1		2	
		n	%	n	%	n	%	n	%
Ovary	22	12	54	4	33	8	67	0	0
Peritoneum	16	14	87	6	42	4	29	4	29
Total	38	26	68	10	39	12	46	4	15

Table IV. Response to therapy and site of biopsy after a 2-month therapy

Site of endometriosis	Total No. of biopsy specimens	Positive biopsy specimens		Morphologic response to therapy					
				0		1		2	
		n	%	n	%	n	%	n	%
Ovary	11	9	82	1	11	7	78	1	11
Peritoneum	21	16	76	0	0	9	56	7	44
Total	32	25	78	1	4	16	64	8	32

Table V. Comparison of the effect on peritoneal implants between 4-month therapy and 2-month therapy

Morphologic response to therapy (grade)	4 mo gestrinone or danazol therapy		2 mo gestrinone therapy	
	n	%	n	%
0	6	42	0	0
1	4	29	9	56
2	4	29	7	44

Table VI. Correlation between change in laparoscopic evaluation of grade of endometriosis according to revised American Fertility Society classification and morphologic response in peritoneal foci at end of therapy

Change in classification	No. of cases	Morphologic response		
		Grade 0	Grade 1	Grade 2
Improvement*	5	2	2	1
No improvement	8	5	1	2

*Defined as a decrease of five points or more according to the revised American Fertility Society classification system (1985).

morphologic response to therapy and the scoring of the response during the menstrual cycle before therapy (Fig. 4). The change in the laparoscopic staging of endometriosis according to the revised American Fertility Society classification system at the end of therapy also did not correspond with the morphologic response to therapy (Table VI).

Comment

Both danazol[2, 3] and gestrinone[4, 5] therapies may induce cellular inactivity and degeneration. This type of cellular involution is substantiated by increased numbers of lysosomes and autophagic vacuoles as well as nuclear changes including deep indentations of the nuclear membrane, condensation of the chromatin, and pyknosis. Endometriotic epithelial cells revealing this type of cytoplasmic and nuclear involution become necrotic and are shed from the basement membrane. Thus this type of involution results in a decreased cell number. Sometimes, however, only a minority of epithelial cells show the nuclear degenerative changes, while cytoplasmic disintegration due to enhanced lysosomal activity is the most common finding for grade 2 therapy response.

Another type of cellular change during medical therapy is characterized by cytoplasmic lipid overload without other obvious cellular degeneration. This second type of change was seen only after danazol therapy.

At the end of a 4-month therapy the first type of degeneration was seen after either danazol or gestrinone, and the second type only after danazol. Cellular involution with cytoplasmic and nuclear damage may

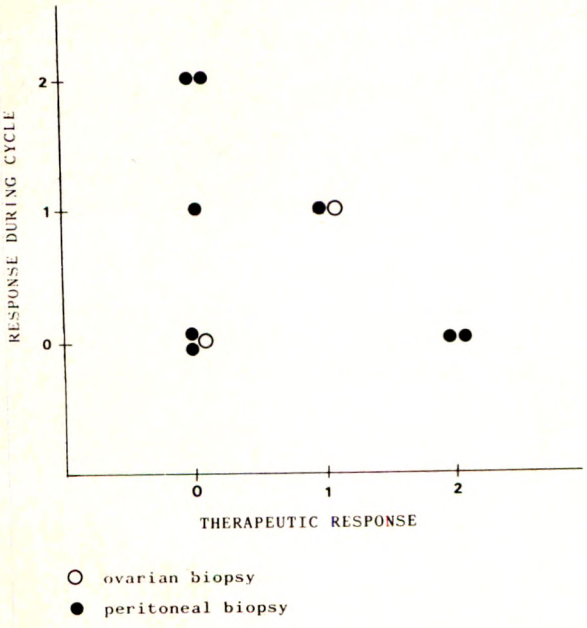


Fig. 4. Correlation between the morphologic response during the menstrual cycle and during therapy in 10 patients with biopsies taken from the same site before and at the end of therapy.

result in cellular death and extrusion of cell remnants into the glandular lumina. Within the cell cytoplasm an enhanced lysosomal activity is seen, while mitochondria, profiles of endoplasmic reticulum, and the perinuclear cisternae usually are dilated and disorganized. Since only this type of involution affects nuclear morphologic features and cytoplasmic organization and finally may result in pyknosis, cellular fragmentation, and extrusion of cell nuclei, it may be concluded that only this first type of cellular degeneration can eliminate individual endometriotic epithelial cells. On the other hand, the presence of large numbers of lipid droplets in ciliated and nonciliated epithelial cells as well as in stromal and perivascular cells induces no dramatic nuclear damage; therefore, this cellular change probably cannot be regarded as lethal.

The present comparative study indicates that a 2-month therapy with gestrinone (1.25 mg daily) can induce a degree of cellular inactivation and regression in peritoneal endometriotic implants that is more pronounced than after a 4-month therapy with either gestrinone (2.5 mg twice or thrice weekly) or danazol (600 mg daily). Furthermore, our morphologic findings are in agreement with the clinical observation that ovarian implants of endometriosis are more resistant to medical therapy than are peritoneal implants but do not support the hypothesis that prolonged medical therapy with either danazol or gestrinone can eradicate endometriotic implants. Cellular involution and degradation are seen in approximately 25% of the implants and this effect is apparently not increased with prolongation of the treatment. On the contrary, if the dose is increased, a 2-month therapy with gestrinone can produce a stronger effect on the implant than can a 4-month therapy.

The advantages of a short-term medical therapy of endometriosis in infertility patients are obvious but the clinical value needs further investigation. On the other hand, the rationale for long-term medical therapy of endometriosis in infertility has never been clear. Although long-term therapy is frequently required for the treatment of pelvic pain in endometriosis there are no reasons to assume that the mechanisms of pain and infertility in endometriosis are related to each other. A beneficial effect of pregnancy on endometriosis has been proposed as a model for hormonal therapy and a long-term estrogen-progestogen therapy was introduced by Kistner.[10] However, the beneficial effect of pregnancy on endometriosis has been questioned and the remarkable involutionary processes during the postpartum period are as likely to cause regression of endometriosis as is the hormonal status of a 9-month gestation. Laparoscopic observation has shown regression and involution of endometriotic implants after long-term medical therapy. However, laparoscopic evaluation of endometriosis can be misleading in the evaluation of the effect on the implants in terms of tissue. Laparoscopy reveals the sequelae of endometriotic activity such as secretion, hemorrhage, inflammatory reaction, or complications such as adhesions and endometrioma formation but not the implant itself.[11] When laparoscopic evaluation of the therapeutic effects is performed at the end of or immediately after therapy, the ovarian function is still suppressed and the effect of treatment is compared under two different hormonal conditions. Inactivation of the endometriotic implant may allow regression of the secretory activity, hemorrhage, and inflammatory reaction. However, regression and resorption of the implant in terms of cells can be verified only by morphologic examination of the suspected focus. If laparoscopy is used as the method for evaluation of the therapeutic effect, it should be planned by preference not at the end of therapy but during one of the cycles after treatment, when the ovarian function has been restored.

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Cord sampling for the evaluation of oxygenation and acid-base balance in growth-retarded human fetuses

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In 14 pregnancies complicated by intrauterine growth retardation, the umbilical cord was sampled before delivery under ultrasonic guidance for rapid fetal karyotyping. Fetal blood was analyzed for respiratory gases, acid-base balance, and lactate concentrations. Two patients were excluded from the study because cord samples were diluted with amniotic fluid. In six patients (group 1), the clinical assessment warranted continuation of pregnancy. Cesarean sections were performed in the remaining eight patients (group 2) within 8 hours of cord sampling. The data from the two groups were compared with those obtained from umbilical venous blood at the time of elective repeat cesarean section in term appropriate for gestational age infants (controls). No significant difference in Po_2 was found between groups 1 and 2 and controls. In contrast, there were significant differences in oxygen saturation and acid-base balance between groups 1 and 2. Lactate concentration was inversely correlated with pH and was elevated in five of six fetuses requiring a prompt cesarean section: In two of these five fetuses, nonstress fetal heart rate tracings were reactive. The results suggest that fetal blood biochemistry, and particularly lactate concentration, may represent an additional indicator of fetal well-being in pregnancies complicated by intrauterine growth retardation. (Am J Obstet Gynecol 1987;157:1221-8.)

Key words: Cord blood sampling, lactate concentration, intrauterine growth retardation, respiratory gases, acid-base balance

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Recently, techniques have been developed for the sampling of fetal blood from the umbilical cord of human fetuses using the technique of fetoscopy for visualization of the cord. This approach has been extensively used for genetic diagnosis in second-trimester fetuses.[1] More recently, improvements in techniques for ultrasonic visualization of the umbilical cord have permitted direct sampling of fetal blood from third-trimester fetuses from the cord by means of a needle guided by ultrasonography.[2, 3] We were interested in

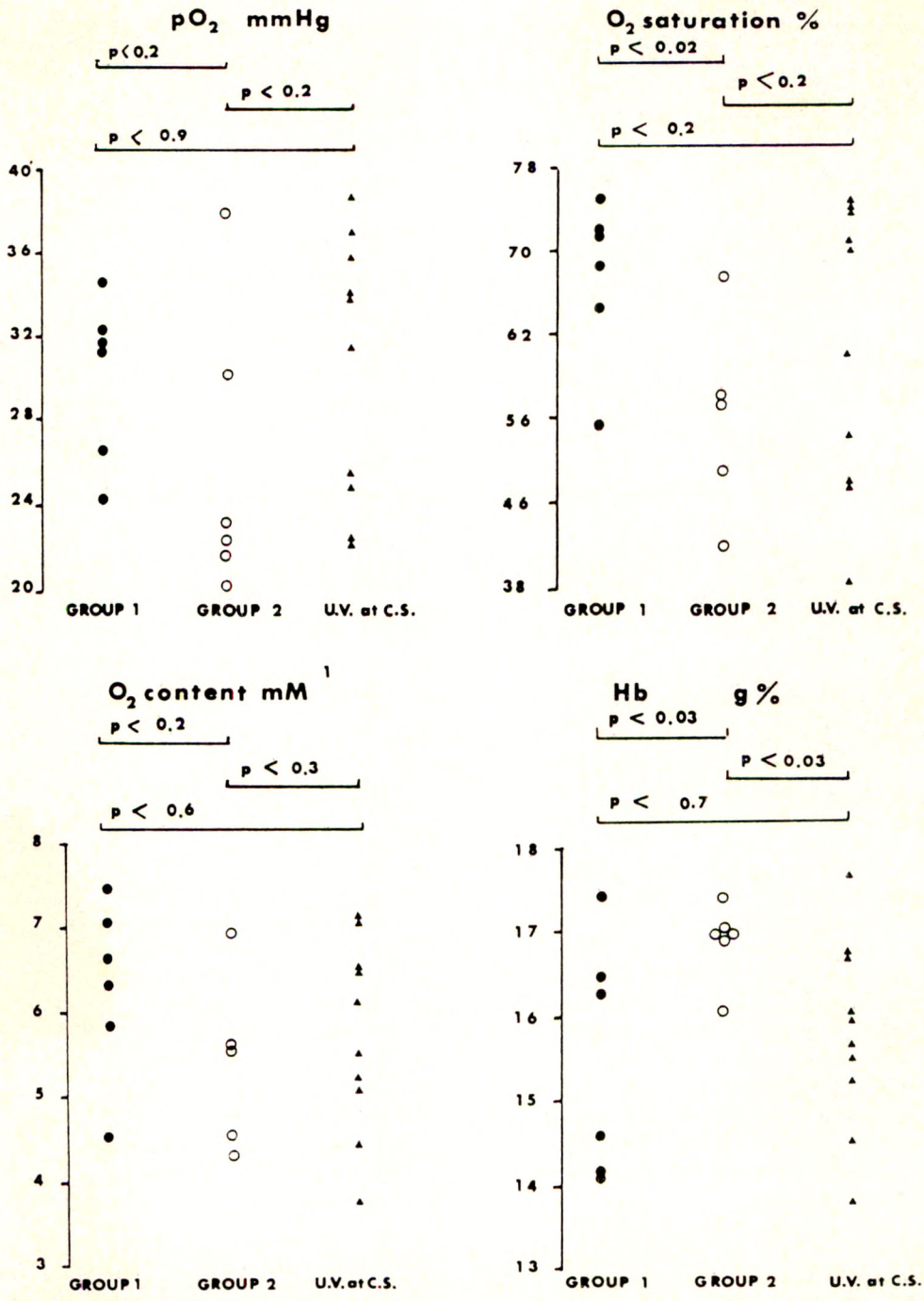


Fig. 1. Comparisons of Po_2, oxygen saturation, oxygen content, and hemoblogin concentration between groups 1 (●) and 2 (○) and between the two groups and the control group (elective cesarean sections ▲). (*U. V.* = Umbilical venous; *C. S.* = cesarean section.)

pursuing the evaluation of fetal blood obtained by this approach in infants with fetal growth retardation. Fetal growth retardation is a fairly common problem in high-risk obstetrics and can be reliably diagnosed. However, no effective therapy in utero is yet available. Clinical management relies on the best timing of delivery to avoid severe fetal distress with its attendant neonatal complications. Although the timing of delivery has relied primarily on the interpretation of antepartum fetal heart rate testing and biophysical profile, both have limitations. Thus additional methods are needed to evaluate fetal well-being.

In this study we have performed umbilical cord blood sampling in 14 pregnancies complicated by fetal growth

Table I. Clinical information about patients in groups 1 and 2

Case No.	*Weeks (sample)*	*Time sample/delivery*	*Delivery*	*Sex and fetal weight (gm)*	*Percentile*	*Apgar score*	*Outcome/discharge*
1	38	6 days	CS	M, 2500	<5	4-9	Alive, 5 days
2	38	7 days	VD	F, 2800	20	7-9	Alive, 5 days
3	32	10 days	CS	F, 1220	<2	6-10	Alive, 13 days
4	34	25 days	CS	M, 2000	<2	5-8	Alive, 10 days
5	37	12 days	VD	M, 2700	<5	8-10	Alive, 5 days
6	32	25 days	CS	M, 1750	<2	7-10	Alive, 37 days
7	37	8 hr	CS	F, 2300	<10	2-5	Alive, 8 days
8	31	2 hr	CS	F, 710	<2	5-8	Died, 3 days
9	31	2½ hr	CS	M, 1150	<5	4-8	Alive, 53 days
10	29	2 hr	CS	F, 560	<2	2-2	Died, 1 day
11	32	3 hr	CS	F, 1150	<2	8-9	Alive, 57 days
12	35	1 hr	CS	F, 1700	<2	4-7	Alive, 28 days

CS = Cesarean section; VD = vaginal delivery.

retardation for rapid karyotyping. The blood samples have also been analyzed for respiratory gases, acid-base balance, and lactate concentrations, and these values were compared with data obtained from umbilical venous blood in 10 normal pregnancies at the time of elective cesarean section.

Material and methods

Patients. Fetal growth retardation was diagnosed in 14 pregnant women by ultrasound measurements of biparietal diameter and cephalic and abdominal circumferences. Gestational age was assessed by the last menstrual period and an ultrasound determination within 20 weeks of pregnancy. Fetal growth retardation was confirmed in all cases after birth by reference to Italian birth weight–gestational age standards.[4] The main indication for fetal blood sampling was rapid fetal karyotyping, since fetal growth retardation is often associated with chromosomal abnormalities.[5] The 14 fetuses reported in this study showed a normal karyotype and no malformations at birth. In 12 of the 14 fetuses, a late flattening of growth was identified whereas two fetuses had a low profile growth curve and marked oligohydramnios. At the time of fetal blood sampling, gestational age ranged from 29 to 38 weeks. Informed consent was obtained from all patients.

In six of the 14 patients, delivery occurred 1 to 3 weeks after cord sampling (group 1). In the other eight patients, a cesarean section was performed within 8 hours of sampling (group 2). Fetal blood biochemical values, even though they were known to the clinical staff, did not primarily influence the timing of delivery. The clinical decision regarding whether the pregnancy should be allowed to continue was based on a multiplicity of factors including the maternal clinical condition, gestational age, antepartum fetal heart rate testing, sonographic evaluation of fetal growth, fetal body and breathing movements, and amniotic fluid volume. In Table I clinical information about the two groups is presented in detail; two samples were judged to be diluted by amniotic fluid (Table II) and were excluded from data analysis.

The control group was made up of 10 patients undergoing an elective cesarean section. All patients had an uneventful pregnancy and the newborns' weights were appropriate for gestational age.[4] At delivery gestational age ranged from 35 to 39 weeks.

In all cases the cesarean section was performed under general anesthesia. Patients were placed in the left lateral recumbent position and received 7 $ml \cdot kg^{-1} \cdot hr^{-1}$ of NaCl 0.9% intravenously. Maternal blood pressure was monitored, and in no instance was hypotensive episodes of greater than 40 mm Hg detected. Immediately after fetal extraction, the cord was doubly clamped, and blood was withdrawn from the umbilical artery and vein.

Technique of umbilical cord blood sampling. The procedure was performed after an overnight fast by means of an aseptic technique: No local anesthetic or intravenous sedative was used. Fetal heart rate testing was recorded before and after the sample.

Fetal blood was sampled from the placental insertion of the umbilical vein by a 20-gauge needle guided by sonography in nine pregnancies according to standard techniques.[2] In four fetuses the sonographic target of the needle was represented by sections of the cord fixed, by the scarcity of amniotic fluid, between fetal parts and the uterine wall. In one fetus a chorionic plate vessel was sampled. In all fetuses the angle between the vessel and the needle was predetermined to approximately 20 to 45 degrees. The sampling procedure never exceeded 10 minutes. Two attempts to insert the needle were necessary in three patients. Sonographic imaging of the site of sampling was obtained by a 5 MHz sector transducer and a pulsed Doppler control of the constant or pulsatile flow of the tip of the needle to verify the venous site of sampling during the procedure. The reliability of fetal sampling was also assessed by com-

Table II. Respiratory gases, acid-base balance, and hemoglobin concentration in fetal and maternal blood samples diluted with amniotic fluid

Case No.		Hemoglobin F (gm/dl)	Hemoglobin M (gm/dl)	pH	P_{CO_2} (mm Hg)	Base deficit (mEq/L)	Lactate concentration (mmol/L)
13	Cord-sample (35)*	8.3	13.5	7.287	34.4	−9.2	—
	Delivery	16.0	13.2	7.298	40.2	−6.4	0.95
14	Cord-sample (28)	4.5	12.9	7.262	42.8	−9.5	4.55
	Delivery	17.2	11.1	7.309	46.9	−3.0	1.2

*Numbers in parentheses refer to days between sampling and delivery.

Table III. Comparison between values obtained during cord samples and from the umbilical vein at the time of delivery in groups 1 and 2

Case No.	Value	pH	P_{CO_2} (mm Hg)	Base deficit (mEq/L)	Lactate (mmol/L)	P_{O_2} (mm Hg)	O_2 saturation (%)	O_2 content (mmol/L)	Hemoglobin (gm/dl)
1	Cord-sample (6)*	7.306	38.5	−6.5	—	31.4	53.7	4.5	14.1
	Delivery	7.264	42.1	−8.2	—	24.6	30.8	2.9	15.8
2	Cord-sample (7)	7.373	37.2	−2.9	0.75	32.4	71.9	7.4	17.4
	Delivery	—	—	—	—	—	—	—	—
3	Cord-sample (10)	7.371	36.6	−3.3	0.96	24.3	64.9	6.3	16.3
	Delivery	7.338	42.3	−3.5	1.0	30.8	60.6	5.3	14.7
4	Cord-sample (25)	7.381	34.4	−3.7	1.0	31.8	75.8	6.6	14.6
	Delivery	7.258	54.1	−4.5	1.4	17.8	26.0	2.7	17.3
5	Cord-sample (12)	7.360	37.6	−3.6	1.4	34.8	71.8	7.1	16.5
	Delivery	—	—	—	—	—	—	—	—
6	Cord-sample (25)	7.367	40.6	−1.5	1.0	26.9	68.1	5.8	14.2
	Delivery	—	—	—	—	—	—	—	—
7	Cord-sample (8)	7.303	39.1	−5.8	1.8	30.1	67.4	6.8	17.1
	Delivery	7.252	53.4	−4.2	1.75	22.4	43.1	3.8	15.1
8	Cord-sample (2)	7.264	52.5	−4.3	2.7	38.0	—	—	16.1
	Delivery	7.125	65.7	−9.0	3.75	17.5	49.2	4.0	14.9
9	Cord-sample (2½)	7.320	43.2	−4.1	1.15	21.8	54.9	5.5	16.8
	Delivery	7.288	45.3	−5.4	1.65	25.3	57.8	5.8	16.8
10	Cord-sample (2)	7.302	47.6	−3.2	1.95	22.4	54.5	5.6	17.06
	Delivery	7.155	69.0	−7.7	2.45	17.7	31.7	3.3	17.2
11	Cord-sample (3)	7.283	45.35	−5.3	2.4	20.1	42.0	4.3	16.95
	Delivery	7.257	54.7	−4.3	—	15.6	34.95	3.4	16.4
12	Cord-sample (1)	7.281	48.1	−5.06	1.63	23.3	42.2	4.5	17.8
	Delivery	—	—	—	2.35	—	—	—	—

*Numbers in parentheses refer to days in group 1 and hours in group 2 between sampling and delivery.

Table IV. Mean values ± SEM for the biochemical parameters examined in the three groups

	Group 1	Group 2	Controls
P_{O_2} (mm Hg)	30.3 ± 1.6	26.0 ± 2.8	35.0 ± 2.0
O_2 saturation (%)	67.7 ± 3.2	52.2 ± 4.7	60.8 ± 4.3
O_2 content (mmol/L)	6.3 ± 0.4	5.3 ± 0.4	5.7 ± 0.4
Hemoglobin (gm/dl)	15.5 ± 0.6	17.0 ± 0.2	15.8 ± 0.3
pH	7.360 ± 0.01	7.292 ± 0.01	7.316 ± 0.01
P_{CO_2} (mm Hg)	37.5 ± 0.8	46.0 ± 1.9	45.1 ± 1.7
Lactate (mmol/L)	1.0 ± 0.1	1.8 ± 0.2	0.8 ± 0.04
Base deficit (mEq/L)	−3.6 ± 0.7	−4.6 ± 0.4	−3.9 ± 0.5

parison of maternal and sample hematocrit and hemoglobin concentrations and by analysis of red cell volumes with a Coulter counter. In addition, Kleihauer smears were prepared to confirm the fetal source of blood. After sampling, the cord was continuously visualized by ultrasonography until fetal bleeding ceased. In only one patient cord bleeding persisted more than 60 seconds: After the sample, fetal heart rate underwent a transient mild tachycardia of 170 bpm. In this patients (Table III, No. 7), delivery occurred by cesarean section 2 hours later: Cord hemoglobin concentration decreased from 17.1 to 15.1 gm/dl.

Po_2 (mm Hg)	O_2 saturation (%)	O_2 content (mmol/L)
26.2	60.1	2.9
29.1	60.9	5.8
29.1	56.3	3.0
30.3	59.2	6.0

Table V. Lactate concentration and nonstress fetal heart rate testing in groups 1 and 2

	Lactate (mmol/L)	
Fetal heart rate	<1.5	>1.5
Reactive	6	2
Nonreactive	—	3

Analytic techniques. Blood for all analyses was collected into heparinized syringes that were immediately sealed and stored on ice; the determinations of lactate concentrations and respiratory gases were carried out within 5 to 10 minutes. Care was taken to treat all blood samples as anaerobically as possible. Hemoglobin concentration and oxygen saturation were determined with a Radiometer OSM-2 oximeter. Oxygen content was calculated from these values by the following equation: Oxygen content (mmol/L) = hemoglobin (gm · L^{-1}) × oxygen saturation × 0.05982. pH, Pco_2, and Po_2 were determined on a Radiometer ABL analyzer. Base deficit was calculated from these data. Lactate concentration was measured with a Yellow Springs analyzer (23L). All biochemical analyses were made in duplicate.

Statistical methods. An unpaired Student *t* test was used to calculate the significance of the difference among groups. Linear regression analyses were carried out by the least squares method.

Results

Reliability of the cord samples. None of the 14 samples was contaminated by maternal blood as confirmed by the Kleihauer smears that showed 100% fetal cells. Two samples, the second and the third in chronologic order of the series, were judged contaminated by amniotic fluid because the hemoglobin concentration was 8.3 and 4.5 gm/dl, respectively, whereas the concentration in umbilical venous blood at delivery was 16.0 and 17.2 gm/dl, respectively. In these two fetuses respiratory gases and acid-base values (Table II) were considered unreliable and excluded from data analysis.

The other 12 cord samples were judged uncontaminated by amniotic fluid by the comparison of hemoglobin concentrations of the cord with delivery samples.

Respiratory gases and acid-base values in cord blood. Biochemical data of the 12 reliable cord samples are reported in Table III together with data obtained from umbilical venous blood at delivery. Fig. 1 presents the Po_2, oxygen saturation, oxygen content, and hemoglobin concentration in the cord blood for the two groups of patients. Similarly, Fig. 2 presents the pH, Pco_2, lactate concentration, and base deficit. In both figures the data are compared with those obtained in the control group. Table IV presents the mean values ± SEM for all the biochemical parameters studied in the three groups.

Lactate versus pH and antepartum fetal heart rate testing. Lactate concentrations and pH values for all umbilical cord blood samples showed a significant negative linear relationship ($p < 0.001$) (Fig. 3). On the contrary, no relationship was found between lactate concentrations and base deficit.

Table V presents a comparison of lactate concentrations with nonstress fetal heart rate testing recorded within an hour after the procedure was completed. For lactate concentrations higher than 1.5 mmol/L (five of six patients in group 2), fetal heart rate was reactive in two fetuses.

Comment

The introduction of cord blood sampling under ultrasound guidance during the third trimester presents the obstetrician with an unique opportunity to evaluate the fetus biochemically in a relatively undisturbed condition. It is conceivable that the addition of such information in conjunction with antepartum fetal monitoring may increase the precision with which fetal well-being can be determined. The technique of cord blood sampling required the facilities of a perinatal intensive care center together with a skilled perinatology team, including an expert ultrasonographer. Although this technique is invasive, we have not noticed any serious maternal or fetal complication thus far. This includes 22 other patients in whom the procedure was performed for indications other than fetal growth retardation. The safety of cord sampling has also been reported in two previous papers.[2, 3]

A prime requisite for proper interpretation of the sample is that it be fetal blood uncontaminated by maternal blood or amniotic fluid. In the present study, contamination with maternal blood never occurred. Unfortunately, the Coulter counter cannot be used to differentiate fetal from maternal blood during the last 10 weeks of gestation as it can at 20 weeks, since the difference between fetal and maternal red cells volume is much less. However, the Kleihauer smear is able to discriminate fetal from maternal blood, although the

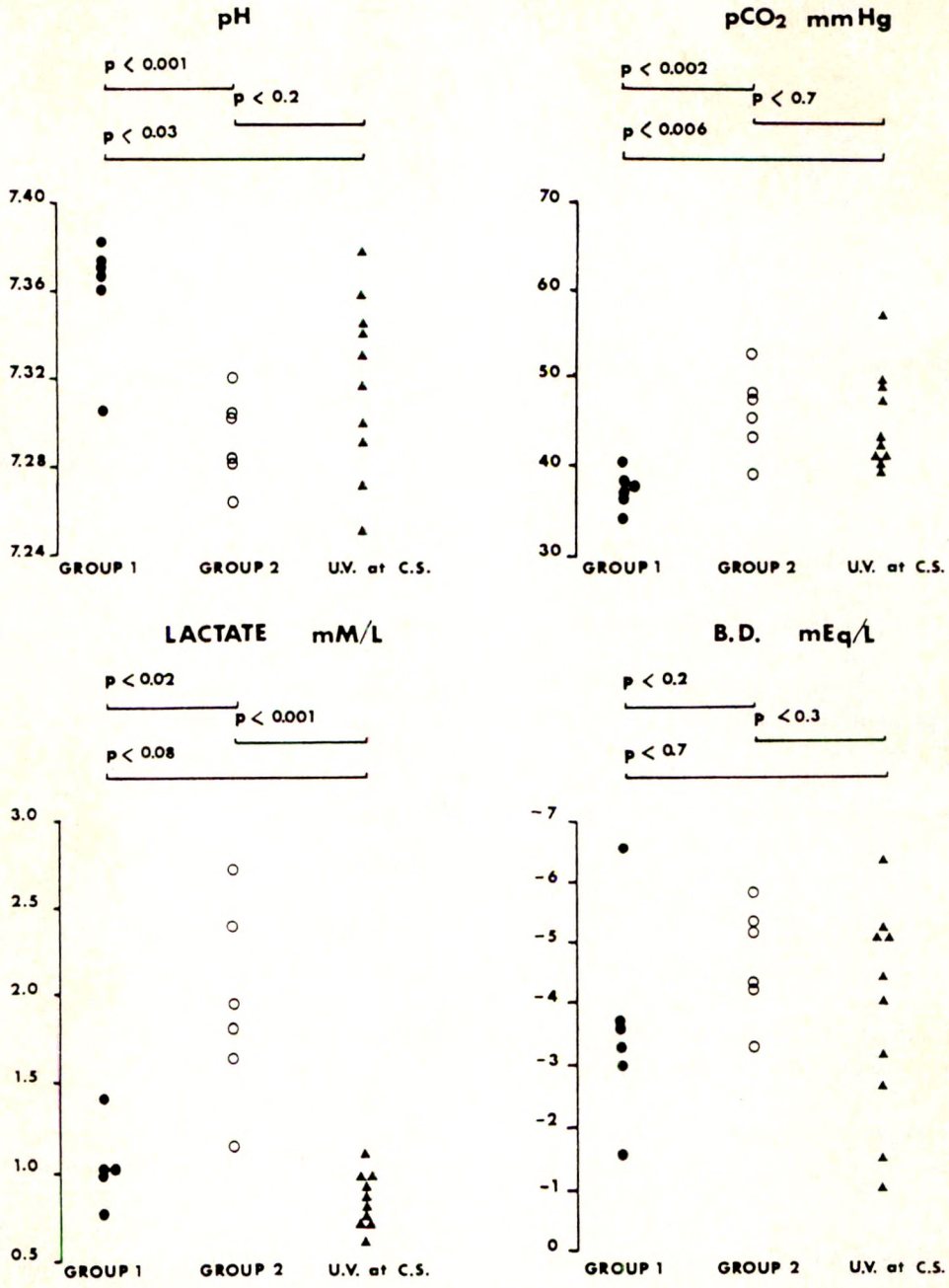


Fig. 2. Comparisons of pH, P_{CO_2}, lactate concentration, and base deficit between groups 1 (●) and 2 (○) and between the two groups and the control group (▲). (*U. V.* = Umbilical venous; *C. S.* = cesarean section.)

results are not immediately available. Futher methods for rapidly discriminating fetal from maternal blood would be helpful.

A second more vexing problem is that of dilution with amniotic fluid, which must be rapidly identified if the biochemical data are to be used for clinical decisions. Since the pH of amniotic fluid is approximately 7.1 and lactate concentration is much higher than that of fetal blood,[6] contamination by amniotic fluid would decrease blood pH and increase lactate concentration. Such contamination could mistakenly suggest fetal distress. Hemoglobin concentration is also decreased by dilution with amniotic fluid and can provide a crude guide when dilution is fairly marked, but better markers of amniotic fluid contamination need to be developed.

In this study the primary indication for cord blood sampling was rapid fetal karyotyping in growth-retarded fetuses, as well as for the evaluation of fetal oxygenation and acid-base balance. In group 1 (six patients), pregnancy was allowed to continue, whereas in group 2 (six patients), the fetuses were judged to be in jeopardy and an immediate cesarean section was per-

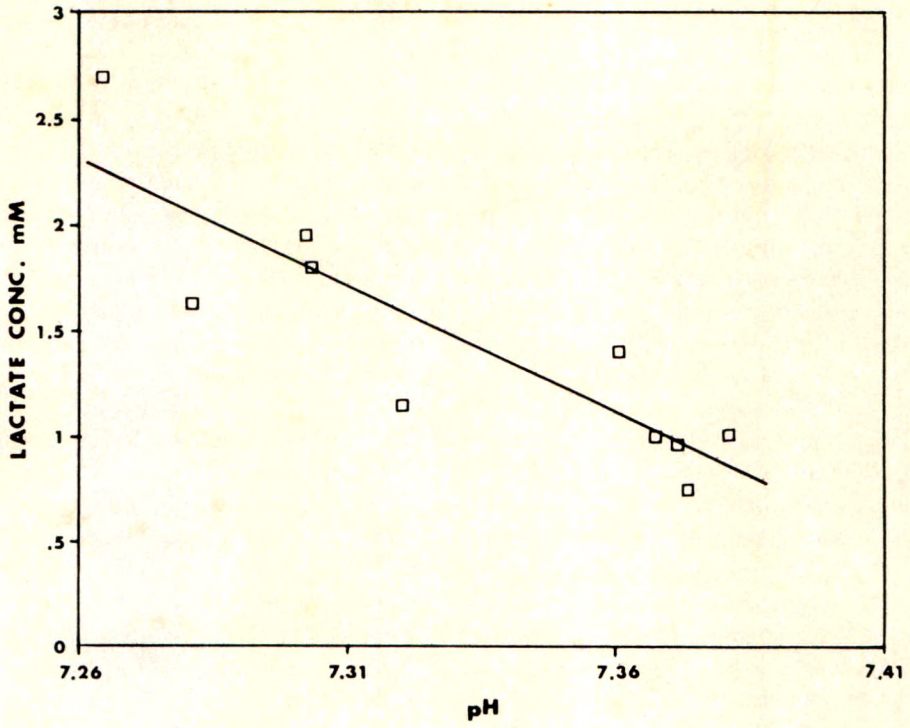


Fig. 3. Lactate concentration versus pH in the cord samples (□ cord samples): $y = 90.3 - 12.1\ x$; $r = 0.88$; $p < 0.0009$.

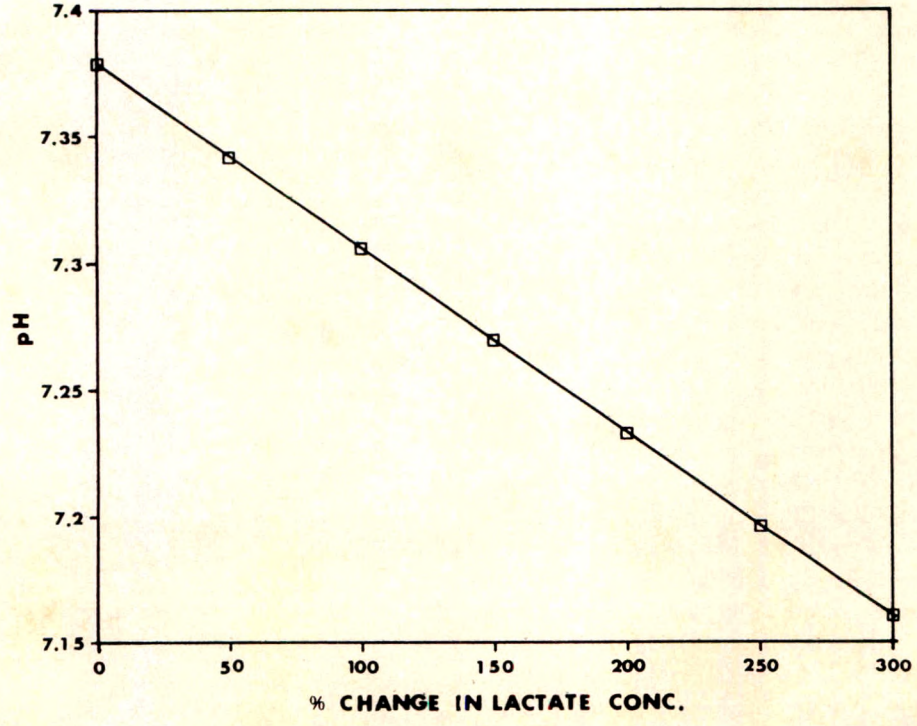


Fig. 4. Cord pH versus percentage change in lactate concentration.

formed. For obvious ethical reasons, normal controls are not available. Thus, we used as a reference the biochemical data obtained from sampling the umbilical cord of normal term fetuses at the time of elective repeat cesarean section, even though the stress of anesthesia and surgery might have affected these values in the sense of a mild acidosis.

An important observation in this study is that the

biochemical parameters in group 1 patients were essentially the same as those in the control group with the exception of a lower P_{CO_2} and a higher pH. On the contrary, major differences were detected between the two groups of growth-retarded fetuses. Despite similar P_{O_2} values, oxygen saturation was decreased in group 2 patients, which presumably reflects lower pH values and the attendant shift in the hemoglobin dissociation curve. The increase in hemoglobin concentrations in group 2 compared with both group 1 and controls explains the lack of difference in oxygen content.

The fact that P_{O_2}, as previously mentioned, was not different in group 2 compared with both group 1 patients and control is rather striking. We believe that this may reflect the fact that umbilical venous blood and not arterial blood was sampled, which suggests that a major problem for the exchange of respiratory gases in small for gestational age infants may be a reduction in umbilical blood flow rather than a reduction in placental-diffusing capacity. Such a hypothesis is consistent with the data obtained by Griffin et al.,[7] who demonstrated an increase in placental resistance in human growth-retarded fetuses that would induce a reduction of umbilical blood flow.

It should be emphasized that the range of P_{O_2} values shown in Fig. 1 for patients in group 2 is very large and not uniformly distributed. As more data accumulate from various medical centers, the identification of a subgroup with lower P_{O_2} values may be feasible. Certainly, one would expect to see a decrease in umbilical venous P_{O_2} in those high-risk pregnancies complicated by a reduction in uterine blood flow, a phenomenon clearly demonstrated in the studies of Wilkening and Meschia[8] in pregnant sheep.

In contrast to the oxygen data, the acid-base data were less equivocal in discriminating group 1 and 2 patients. As shown in Fig. 2, pH was significantly lower and lactate concentration was significantly higher in fetuses delivered by immediate cesarean section compared with the other groups. In our experience a pH lower than 7.30 and a lactate concentration higher than 1.5 mmol/L should be considered bad prognostic indicators. As shown in Fig. 3, fetal blood lactate concentration is inversely related to blood pH. With use of the regression analysis depicted in Fig. 3, Fig. 4 was then constructed to illustrate that changes in lactate concentration may be more sensitive indicators of fetal distress than changes in pH. The mean lactate concentration found in normal patients undergoing elective cesarean section (0.84 mmol/L) was taken as 0% change. Fig. 4 clearly illustrates that an increase in lactate concentration of 100% would still be associated with a pH >7.30.

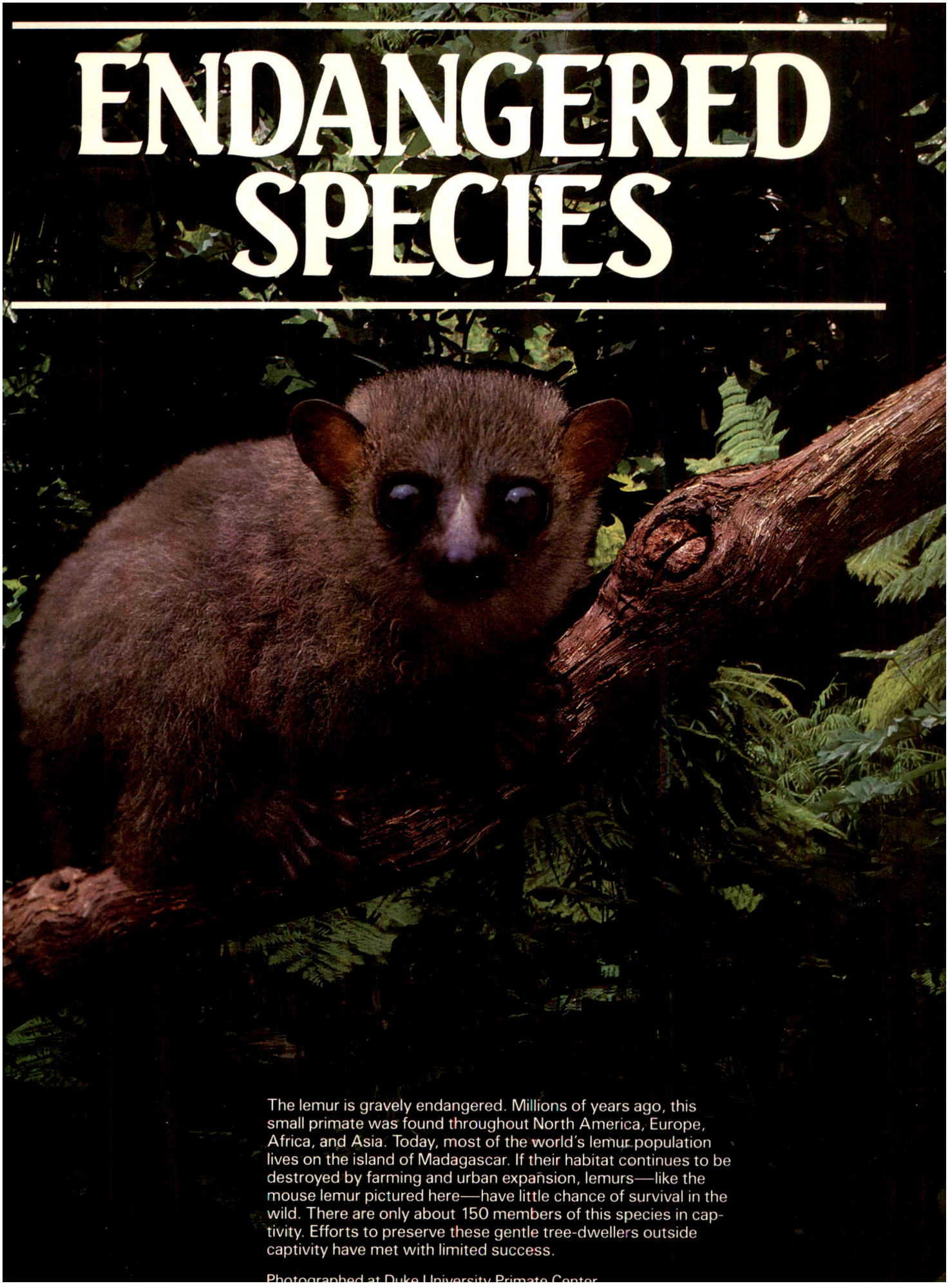
It has been shown in animal species that the normal fetus consumes lactate that is produced by the placenta.[9, 10] Placental production of lactate has also been demonstrated in humans.[11] Under acute hypoxic conditions, the fetus may become a net producer of lactate and fetal blood lactate concentration increases.

It is important to stress that the lactate values presented in this article were obtained with a methodology that permitted completion of the analyses within 5 minutes of sampling. Lactate concentration was always measured first on all samples. It is also important to stress the fact that high lactate concentrations were present in fetuses with normal antepartum fetal heart rate tracings. This might indicate that lactate concentration may represent an earlier indicator of a change in fetal well-being. Certainly, the question of whether fetal blood biochemical analyses would aid in the recognition of fetal distress deserves further confirmation as the technique of cord sampling is more widely used in obstetrics.

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ENDANGERED SPECIES



The lemur is gravely endangered. Millions of years ago, this small primate was found throughout North America, Europe, Africa, and Asia. Today, most of the world's lemur population lives on the island of Madagascar. If their habitat continues to be destroyed by farming and urban expansion, lemurs—like the mouse lemur pictured here—have little chance of survival in the wild. There are only about 150 members of this species in captivity. Efforts to preserve these gentle tree-dwellers outside captivity have met with limited success.

Photographed at Duke University Primate Center

ENDANGERED SPECIES?

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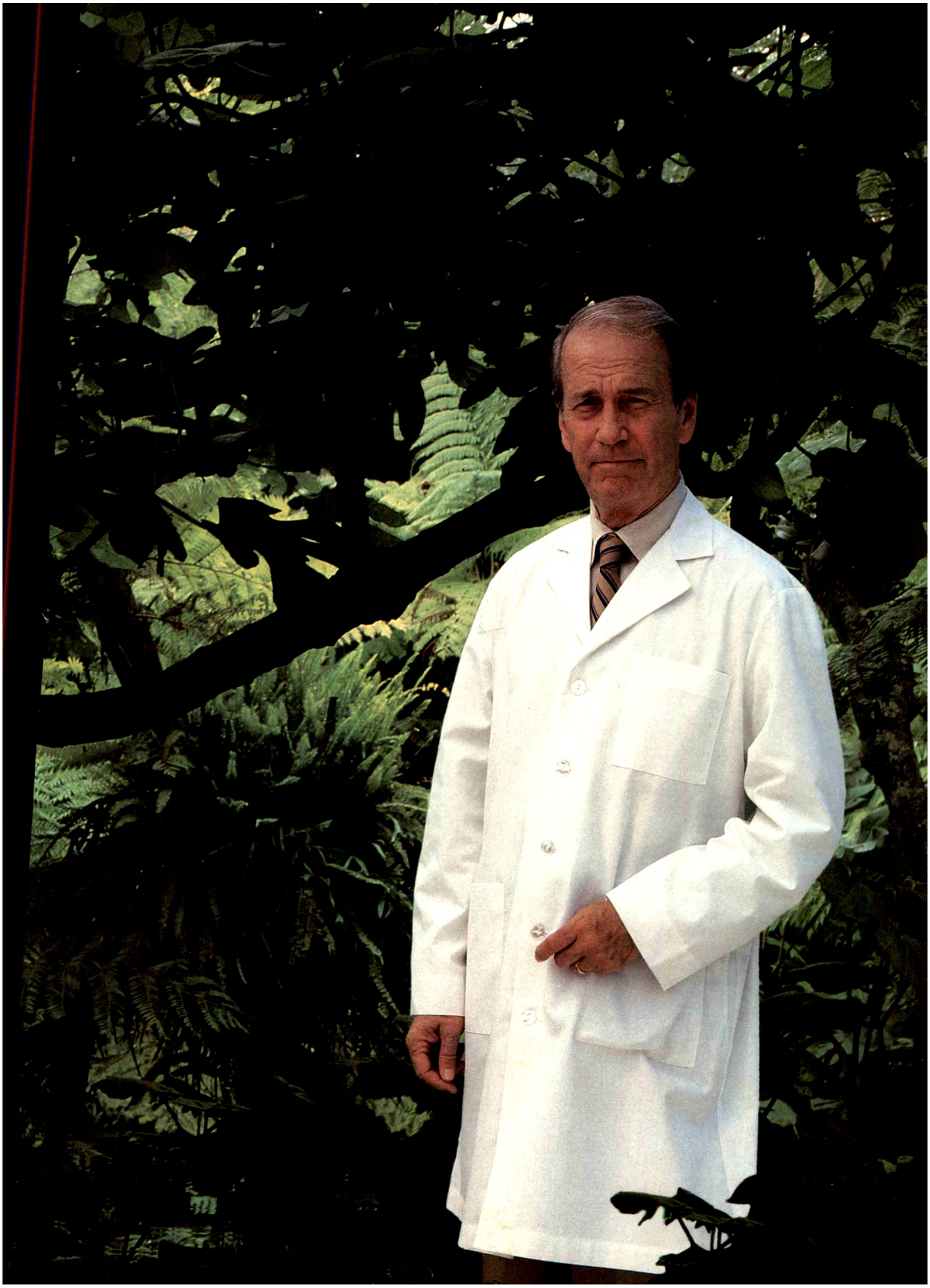
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Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

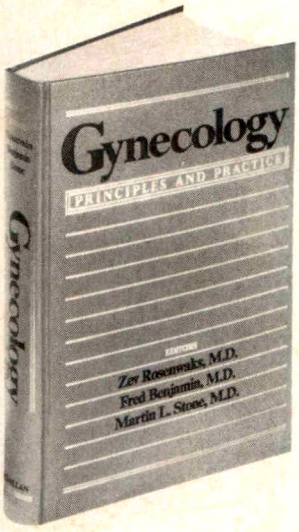
Be alert to earliest manifestations of TE and thrombotic disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. The RCGP prospective study reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. The data suggest varicose veins have little effect on development of deep vein thrombosis but substantially increase risk of superficial vein thrombosis, risk depending on severity of varicosities. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is evidence that estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions (eg, irregular bleeding before OCs, polycystic ovaries) occurre nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer wit combination or progestogen-only OCs, though individual cases were reported. Studies show no increased breast cancer with OCs or estrogen However, greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk of brea cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens rose and further increase with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other risk factors. Decrease in benign brea tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, erosion, and carcinoma in long-term user Cervical microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increased canc risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consid nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of breast canc or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. **4.** *Hepatic adenomas, et* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk, as was age ov 30. Though benign, these lesions may rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk t duration of use, being much greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,00 Consider such lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to half wi acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT ma be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. OCs are contraindicated in case of past present, benign or malignant liver tumors developing during use of estrogenic products. **5.** *Use in or immediately preceding pregnancy; bir defects, malignancy in offspring:* Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in ute to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monit such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DE exposed daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Other estroge may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL for vertebral, anal, cardia tracheoesophageal, renal, and limb defects. There appears preferential expression by males. There was a 4-7-fold increased risk of lim reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threaten abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, includi OCs, during early pregnancy occurred in 18.2/1,000 births, compared to 7.8 for those unexposed (P<.05). More twinning was reported conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but there is considerat evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased abortuses from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion increased soon after stopping OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not be demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods a missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. pregnancy is confirmed, apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy shou use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not u progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 year use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. **7.** *Carbohydrate and lipid metabolism:* Decreasi glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides and tot phospholipids may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few months, and degree may relate to progestog dose. Hypertension prevalence in users is not higher in the first year, but increases with longer exposure; in the 5th year it is 2½—3 tim the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), preexisting ren disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weig gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Ev though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of migraine development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating cause. **10.** *Bleed irregularities:* Breakthrough bleeding (BTB); spotting, and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregu bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology excluded, time or change to another product may suffice. Change to a higher-estrogen OC only when necessary as this may increa thromboembolic risk. Those with history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulat or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulatic possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has be associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients w postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio of ectopic to intrauteri pregnancies is no higher than in nonusers. There may be increased risk of ectopics after discontinuing. **12.** *Breast feeding:* OCs may decrea quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs ur after weaning. **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable differen in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 mont and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete histo and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uteri leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if serious recurrence. Flu retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic, or ren dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholesta jaundice occurred after OC and troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblas anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use wh submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have be observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, I X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyro hormone (PBI, T^4); decreased T^3 uptake, unaltered free T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blo transcortin, corticosteroid, triglyceride, phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influen of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask ons of climacteric. Cervical chlamydial prevalence is increased; do not assume OC protects against chlamydial PID. *Information for patients.* S patient labeling. *Drug interactions:* OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomyc penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidor phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilize tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminoph **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis a thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; coronary thrombosis; cerebral thromb sis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or withc intra-abdominal bleeding, liver cancer, congenital abnormalities. There is evidence of association between the following and OCs, thou confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The followi are believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrh during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargeme secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundi migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted for: premenstrual-like syndrome, catarac libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinit fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hemolytic urem malignant hypertension, itching, vaginitis, porphyria, impaired renal function, acute renal failure, sometimes irreversible, PVCs, abnormal EK pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythematosus, rheumati arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanoma, endometrial, cervical, and brea carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea a withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use a is available on request.**

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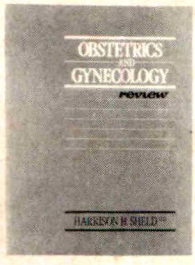
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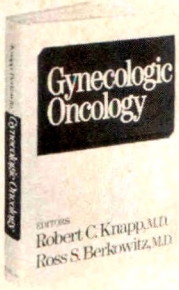
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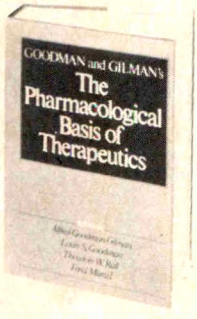
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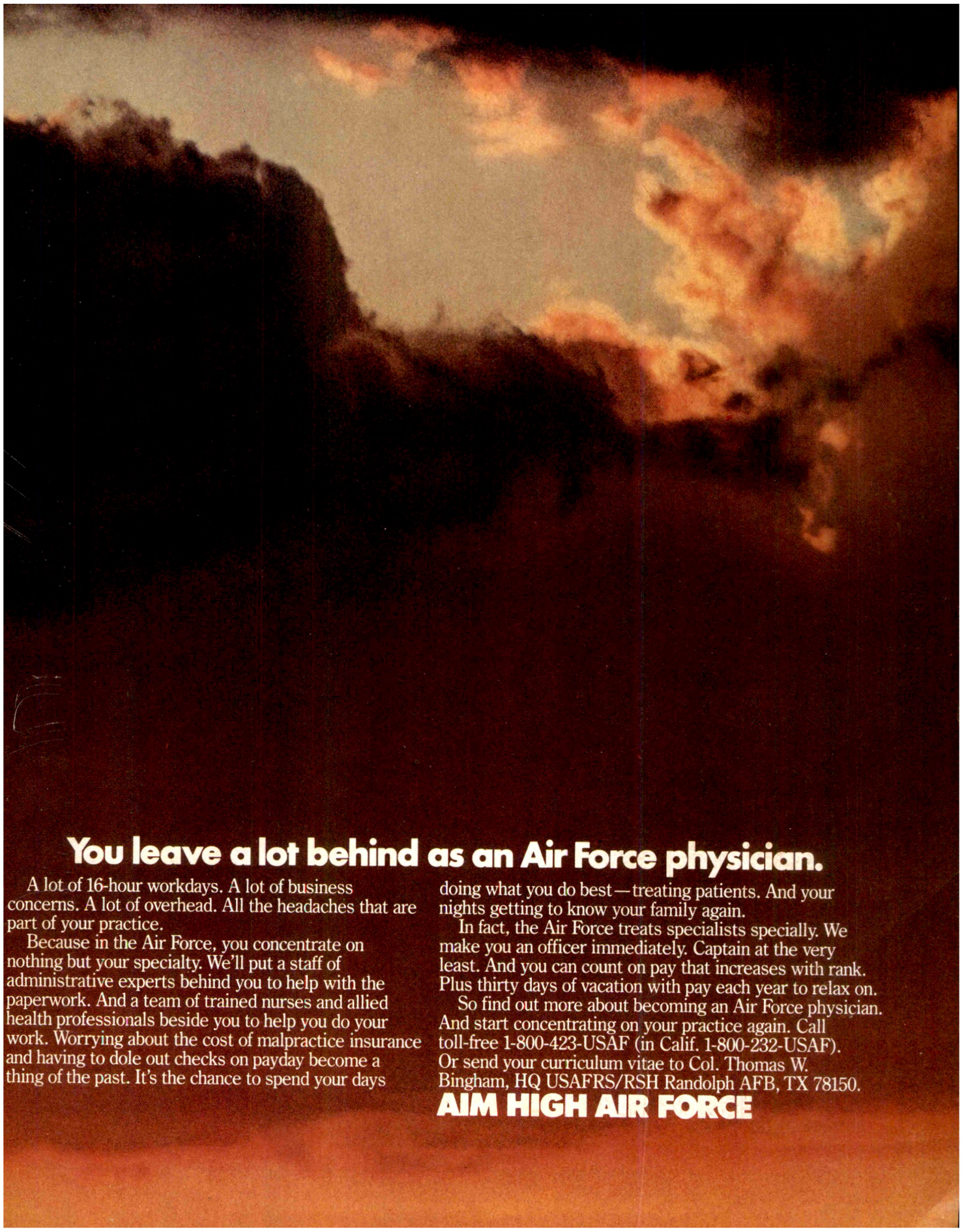
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The length of the human umbilical cord in twin pregnancies

Torgrim Soernes, M.D., and Trygve Bakke, M.D.
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A current hypothesis on the linear growth of the umbilical cord states that cord length is a function of fetal intrauterine motor activity. If there is some type of intrauterine constraint, the tensile forces on the cord will be diminished, and cord length at term will be shorter than if no such constraint is present. One would expect that the voluntary movements of twins would develop under a constraint and that they would have less free space to move compared with single fetuses. If this hypothesis is valid, cord lengths of twins at term would be shorter than those of single pregnancy infants. We measured the cord lengths of 118 infants in 59 twin births and compared them with cord lengths in 9601 single pregnancies. We found that on the average, the umbilical cord length of the twins was 7.90 cm shorter than that of single pregnancy infants. (Am J Obstet Gynecol 1987;157:1229-30.)

Key words: Umbilical cord length, twins, fetal movements

Various reports of recent years[1-4] have pointed toward a new hypothesis concerning the length of the human umbilical cord at term. The hypothesis states that umbilical cord growth occurs as a result of the tensile forces placed on the cord, the most important of which is the movement of the fetus itself.

Experimental studies on animals have shown that if an intrauterine constraint is produced, such as oligohydramnios or fixation of the fetus in one of the uterine horns,[1,2] the fetus will tend to have among other defects a shorter umbilical cord than control animals.

The offspring of animals treated with a paralytic agent[1] during pregnancy, which presumably limits the spontaneous movements of the fetus, also show short cords.

Observations on human infants are few. Strikingly short cords have, however, been found in infants with either evidence of intrauterine compression (early amniotic rupture) or in infants with defects that seriously impair voluntary movements.[3]

We would expect the voluntary movements of fetuses in twin pregnancies to develop under such a constraint inasmuch as their intrauterine space is limited by the other twin. Twin studies, therefore, offer the opportunity to test the hypothesis on normal infants, that is, infants with no serious malformations.

Material and methods

We studied 59 twin births (118 infants) that took place in our hospital between 1979 and 1985. Infants with birth weights below 1000 gm were excluded from the study. The infants had their umbilical cord lengths measured at birth. In our hospital, the cord is routinely cut 2 cm from the fetal abdomen. The umbilical cord is then measured from this point to the placental surface.

The birth weight, birth presentation, and sex of each infant were also recorded.

The lengths of the umbilical cords of singleton infants were also measured, which gave us a control group of 9601 babies of single pregnancies during the same period.[4]

Results were considered statistically significant if $p < 0.001$.

Results

Of the 118 twin infants, 59 were boys and 59 girls.

There were 36 breech presentations and 82 vertex presentations. No statistically significant difference in cord length was found between these two groups.

No significant difference in cord length was found between the two sexes in this study.

The mean cord length of the 118 twins was 49.63 cm, whereas the mean cord length of the 9601 singleton infants was 57.52 cm. This result was statistically significant ($p < 0.001$).

Comment

Previous research[4,5] has demonstrated a sex difference in cord length among singleton infants in that on the average, male babies have slightly longer cords than do female babies. Furthermore, it has been recently shown that there is a tendency for infants born in the breech presentation to have shorter cords than babies born in the vertex presentation.[4]

We did not find any such differences in our twins.

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However, because of the small number of cases in our study, we do not feel that any conclusions can be drawn from our study concerning these points.

The correlation between cord length and birth weight was found to be very small. This is consistent with the results of others.[6-8] Thus we assume that the comparison between twin and singleton cord length can be made regardless of different birth weights.

The salient point of our study is the demonstration that twins seem to have shorter umbilical cords than do single pregnancy infants; on the average the difference is 7.90 cm. At present, the most likely explanation for this is that the presence of the other twin in utero represents a limitation on intrauterine fetal movements.

Thus the results obtained in this study seem to lend credence to the hypothesis that cord lengths at birth can be viewed as a measure of the fetal level of intrauterine activity during the gestational period.

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Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole

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In a controlled, nonrandomized trial a treatment group of 24 multigravid women with a history of at least two previous pregnancies, all complicated by idiopathic fetal growth retardation and placental infarction, received 1 to 1.6 mg/kg aspirin and 225 mg dipyridamole daily from 16 to 34 weeks' gestation in a total of 30 pregnancies. The end-point measure of the study was birth weight related to gestational age. Results obtained in the treatment group were compared with those in 27 pregnancies of a control group of 24 multigravid women with a similar history of recurrent fetal growth retardation who received comparable antenatal care without low dose aspirin and dipyridamole. Fetal growth retardation occurred in 61% of the control pregnancies and in only 13% of treated pregnancies; severe fetal growth retardation was not observed in treated pregnancies, but it occurred in 27% of the control group. In treated women, platelet cyclo-oxygenase activity was suppressed to 5% to 10% of its pretreatment level, but no effect on vascular prostacyclin production was demonstrated. Treatment did not produce adverse effects in mothers or infants. Low-dose aspirin and dipyridamole direct prostacyclin/thromboxane A_2 balance in pregnancy to the dominance of prostacyclin and may thus prevent idiopathic uteroplacental insufficiency and fetal growth retardation in high-risk patients. (Am J Obstet Gynecol 1987;157:1230-5.)

Key words: Recurrent idiopathic fetal growth retardation, low-dose aspirin, dipyridamole, thromboxane, prostacyclin

Idiopathic fetal growth retardation, i.e., not attributable to fetal abnormality, maternal disease, or complications of pregnancy, is associated with reduced uteroplacental perfusion,[1] abnormal platelet behavior,[2,3] and uteroplacental arterial thrombosis resulting in placental infarction.[4] These pathologic processes have been explained by abnormal platelet-vessel and platelet-platelet interactions caused by an insufficient production of prostacyclin, resulting in a relative dominance of thromboxane A_2 in the uteroplacental cir-

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culation.[5] Thromboxane A_2, a powerful vasoconstrictor and stimulator of platelet aggregation, is the main cyclo-oxygenase metabolite of arachidonic acid in platelets and is also produced by various pregnancy-associated tissues.[6] A daily dose of aspirin as low as 1 to 2 mg/kg effectively inactivates platelet cyclo-oxygenase and inhibits platelet thromboxane A_2 synthesis, whereas the production of vascular prostacyclin, which has potent and opposite effects on vascular tone and platelet function, remains almost unaffected.[7] Low-dose aspirin may thus direct prostacyclin/thromboxane A_2 balance to the dominance of prostacyclin, an effect that may be potentiated by dipyridamole.[8] These considerations induced us to test the hypothesis that early antiplatelet therapy with a low dose of aspirin and dipyridamole may prevent uteroplacental thrombosis, placental infarction, and idiopathic fetal growth retardation in pregnant women judged to be at high risk on the basis of a history of recurrent severe fetal growth retardation.

Material and methods

General design. Initially we intended to perform a randomized, placebo-controlled trial, but we discovered at an early stage that patients with a history of recurrent severe fetal growth retardation, often with stillbirths, wanted treatment and were reluctant to take part in a randomized study, even without the use of placebo. It was then decided to use antiplatelet therapy in all patients eligible under previously established criteria. An external control group was composed of pregnant women without antiplatelet therapy who met the same criteria, received the same antenatal care, and were followed in the same hospitals as the treated patients. Primary end points in either group were fetal and neonatal mortality, birth weight, and gestational age at delivery. The study was approved by the hospital ethics committee.

Patients in treatment group. All Caucasian women referred to our outpatient clinic between January 1, 1983, and July 1, 1986, with a history of at least two pregnancies of more than 20 weeks' duration, all complicated by severe fetal growth retardation and placental infarction, without any other known complication of pregnancy or associated disease, were invited to take part in the study. None of the patients refused, and all gave their informed oral consent. The study was terminated when the treatment group included 24 patients, the same number as the control group. The obstetric history of these women included 80 singleton pregnancies, 20 of which were lost before 16 weeks' gestation. The remaining 60 pregnancies had resulted in delivery of growth-retarded babies, with the majority of birth weights below the 2.3 percentile of the distribution of normal weight for gestational age, corrected for parity and fetal sex (Table I). Only 11 babies were born alive, and 10 survived. No fetal malformations were observed, and all placentas showed extensive infarction.

Table I. Obstetric history of 48 patients with recurrent idiopathic fetal growth retardation

	Treatment group (n = 24)	*Control group (n = 24)*
No. of women		
Para 2	16	17
Para 3	4	4
Para 4	4	3
No. of pregnancies*	80	60
With spontaneous abortion	20 (11)	10 (5)
With fetal death	49 (24)	7 (5)
With neonatal death	1	1
With birth weight in relation to gestational age		
<2.3rd percentile	38 (22)	16 (13)
2.3rd-5th percentile	14 (11)	20 (14)
5th-10th percentile	8 (6)	14 (12)

*Number of events, with number of different patients in parentheses.

None of the women had a history of hemostatic disorders or thromboembolic disease. General physical examination before pregnancy revealed no abnormalities except mild and untreated essential hypertension in four women. Results of determination of the activated partial thromboplastin time, the dilute prothrombin time, and the kaolin clotting time suggested the presence of lupus anticoagulant[9] in two patients; cardiolipin antibody levels were not measured. Other laboratory tests did not reveal evidence of autoimmune, coagulation, or hepatic disorders or renal disease. The platelet life span was estimated by a nonradioisotopic technique[2] and was found to be normal (7 to 10 days) in all women.

Treatment protocol. After the women had become pregnant, the presence of a live fetus was confirmed by sonography at 12 to 14 weeks' gestation. The above tests were repeated. From 16 weeks' gestation onward all patients received one low dose of plain aspirin daily (1 mg/kg/day, rounded off to the nearest 10 mg) and 75 mg dipyridamole, three times daily. The capacity of platelets to synthesize thromboxane A_2 in vitro on stimulation with thrombin was assessed by determination of the production of malondialdehyde.[2] Malondialdehyde production was measured every 2 to 3 weeks, and the dose of aspirin was adjusted to suppress malondialdehyde production to 5% to 10% of its pretreatment value. In addition, the four patients with essential hypertension received methyldopa (750 to 1000 mg/day); no other treatment was given except oral iron supplements, and diets were unrestricted. All patients were explicitly told not to use any other aspirin-containing compounds.

At 28 weeks' gestation the production of prostacyclin

Table II. Course and outcome of subsequent pregnancies in 48 patients with recurrent idiopathic fetal growth retardation

	Treatment group (n = 24)	*Control group (n = 24)*	*p value*
Age (yr, median and range)	30 (21-35)	29.5 (24-36)	NS
No. of pregnancies*	30	27	
With spontaneous abortion	0	1	
With fetal death	1	0	
With neonatal death	0	0	
With birth weight in relation to gestational age			
<2.3rd percentile	0	7 (7)	0.01
2.3rd-5th percentile	2 (2)	5 (5)	
5th-10th percentile	2 (2)	4 (3)	
>10th percentile	26 (22)	10 (8)	<0.002
Duration of pregnancy at delivery (wk, median and range)	37 (27-39)	38 (33-43)	NS
No. of cesarean sections	12 (10)	6 (6)	NS
No. of repeat cesarean sections	7 (5)	1	

*Number of events, with number of different patients in parentheses.

and thromboxane A_2 was assessed by determination in venous plasma of their stable metabolites, 6-oxo-prostaglandin $F_{1\alpha}$ and thromboxane B_2, respectively. Values obtained in the same gestational period from 30 randomly selected pregnant women with uncomplicated pregnancies resulting in the term delivery of live infants with birth weights above the tenth percentile were used as controls. The eicosanoids were measured by radioimmunoassay after solvent extraction with commercial kits (Seragen, Inc., Boston, Massachusetts). The platelet life span was again determined at 32 to 34 weeks' gestation, if the patients were still pregnant, and treatment was then terminated.

None of the patients smoked during pregnancy. Antenatal care included serial sonography and cardiotocographic monitoring of the fetus in the third trimester. All patients remained ambulatory unless obstetric complications occurred. The investigators acted as observers; all clinical decisions were made by the patients' own obstetricians in the four hospitals involved.

Patients in control group. The obstetric records of the University Hospital Dijkzigt and of the affiliated Ikazia Hospital were searched for patients with recurrent idiopathic fetal growth retardation delivered after 20 weeks' gestation between January 1, 1977, and December 31, 1982. We found 68 Caucasian women who had at least two pregnancies and were delivered of growth-retarded babies with infarcted placentas in all of them and whose records indicated that fetal growth retardation could not be attributed to fetal abnormality, complications of pregnancy, or maternal disease. Follow-up data obtained from 64 women showed that 40 had not become pregnant again, whereas 24 women had had one or more subsequent pregnancies between 1983 and 1987. These 24 women composed the control group; the history of their 60 index pregnancies is summarized in Table I. Seven of 50 babies were stillborn. In their subsequent pregnancies the women received the same antenatal care as the women in the treatment group, and in the same hospitals, except the University Hospital Dijkzigt. Various therapeutic regimens were prescribed, including prophylactic antihypertensive medication and bedrest, but they received no aspirin and dipyridamole. Two of the women smoked during pregnancy.

Statistical analysis. Differences in relative frequencies between treatment and control groups were analyzed with the χ^2 or Fisher's exact test. The Wilcoxon rank-sum test was used to analyze differences in measured variables between the two groups. A p value <0.05 was regarded as significant.

Results

Patients. Comparison of both groups shows that the total number of index pregnancies (abortions excluded) and the distribution of parity are similar. However, fetal death and severe fetal growth retardation (<2.3 percentile) occurred significantly more often in the index pregnancies of the treatment group than in those of the control group (Table I). In the treatment group four patients had two and one patient had three subsequent pregnancies, so we report the results of 30 treated pregnancies. In the control group three women had two subsequent pregnancies, resulting in a total of 27 pregnancies (Table II).

Course and outcome of pregnancy. In the control group the number of viable pregnancies complicated by severe fetal growth retardation (<2.3 percentile) was seven (27%) and the total percentage of fetal growth retardation (<tenth percentile) was 61.5. This is in con-

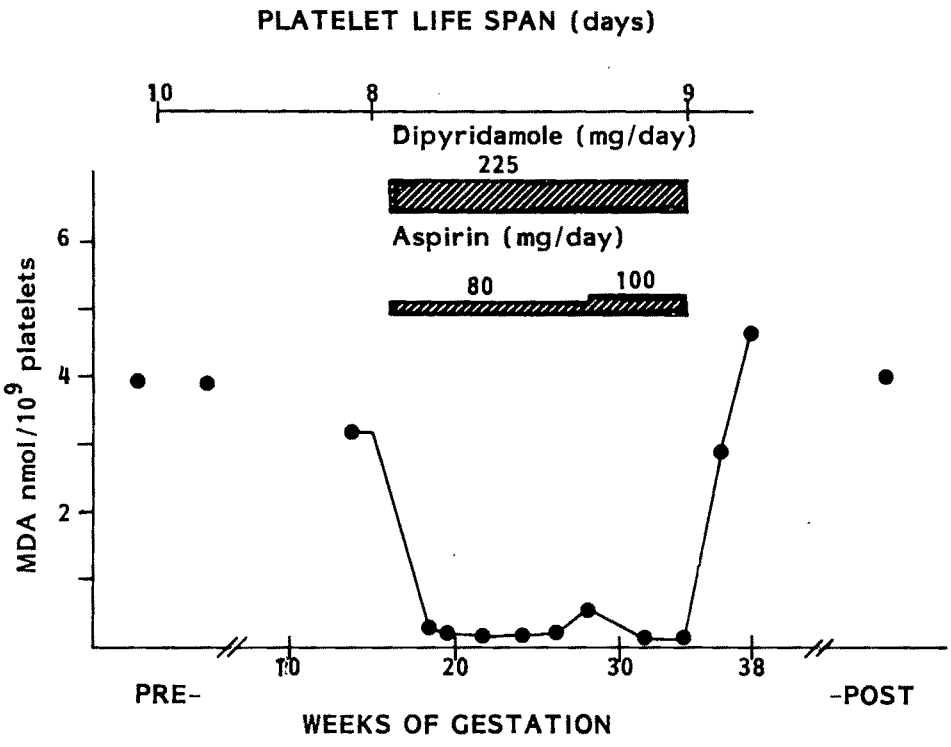


Fig. 1. Platelet life span and malondialdehyde production in one patient before, during, and after pregnancy. This pregnancy ended successfully at 38 weeks.

trast with the results obtained in the 30 treated pregnancies, in which no severe fetal growth retardation occurred and the total percentage of fetal growth retardation was 13.3; the difference is statistically significant ($p < 0.002$). The difference between groups remains significant when only the first subsequent pregnancies are considered. The two infants with birth weights between the 2.3 and fifth percentiles in the treatment group were delivered of the two patients with lupus anticoagulant.

In the treatment group two pregnancies were complicated by mild pregnancy-associated hypertension (diastolic blood pressure 90 to 100 mm Hg) in the third trimester, after treatment had been stopped. In the control group mild hypertension occurred in four pregnancies. In the treatment group small areas of infarction (5% to 10% of placental volume) were found in five placentas, two of which came from the two patients with lupus anticoagulant, and one was delivered by a patient with mild pregnancy-associated hypertension. The placentas of the seven severely growth-retarded infants in the control group were moderately to severely infarcted. Unfortunately, not all placentas of the other infants in the control group were systemically examined.

There was one fetal death in the treatment group, in a 24-year-old woman, gravida 3, with an uncomplicated pregnancy until 27 weeks' amenorrhea, when abruptio placentae occurred. The stillborn baby had a normal weight for gestational age of 950 gm. The median duration of pregnancy was 1 week shorter in the treatment group than in the control group, but this difference is not statistically significant. Because of the high number of repeat cesarean sections performed during labor, the total cesarean section rate was two times higher in the treatment than in the control group (not significant).

In nine pregnancies a reduction of platelet life span of 2 days or more as compared with nonpregnant values was found at 14 weeks' gestation. In seven of these patients the platelet life span could again be determined at 34 weeks, and appeared to have returned to prepregnancy values. The dose of aspirin needed to suppress platelet malondialdehyde production to the desired 5% to 10% of the pretreatment level ranged between 40 and 100 (median 80) mg/day. In 18 pregnancies the aspirin requirement did not change throughout pregnancy, and in 12 pregnancies it increased slightly to a maximum dose of 1.6 mg/kg/day in one patient. The response of platelet malondialdehyde production to treatment in one patient is shown in Fig. 1.

Plasma concentration of 6-oxo-prostaglandin $F_{1\alpha}$ (51.7 ± 9.8 ng/L) in treated patients was not significantly different from control values (47.4 ± 13.5 ng/L), whereas plasma thromboxane B_2 levels in treated

patients (18.6 ± 6.1 ng/L) were significantly ($p < 0.01$) lower than those in controls (38.4 ± 12.2 ng/L).

No adverse effects were noted in the treatment group, and no excessive bleeding was observed during delivery or cesarean section in seven women who still received treatment at that time. None of the infants in either group showed congenital abnormalities. In the treatment group one infant, born at 27 weeks, developed severe respiratory distress but recovered. Periods of moderate respiratory distress occurred in four other premature babies in the treatment group and in three infants in the control group. No other neonatal complications were encountered in either group, in particular no hemorrhagic complications in the treatment group. All infants are thriving.

Comment

A physiologic balance between prostacyclin and thromboxane A_2 appears to be important in the regulation of vascular tone and platelet-vessel and platelet-platelet interactions, not only in the systemic but also in the uteroplacental circulation.[6] An imbalance in favor of thromboxane A_2 may be expected to interfere with the physiologic adaptation of the maternal circulation, which may become clinically apparent as pregnancy-induced hypertension or preeclampsia, fetal growth retardation, or both.[5, 10] The factors that govern prostacyclin synthesis in normal pregnancy have not yet been elucidated, and for that reason it is not known why prostacyclin production may become relatively deficient in some women. It is known, however, that prostacyclin synthesis may be inhibited by a circulating lupus anticoagulant, a condition associated with repeated abortions, fetal growth retardation, and fetal death.[11]

Our results show that a low daily dose of aspirin (1 to 1.6 mg/kg) combined with dipyridamole, 225 mg/day, markedly suppressed platelet thromboxane synthesis, as indicated by a fall in thrombin-induced malondialdehyde production to 5% to 10% of the pretreatment level. Also, the low plasma thromboxane B_2 values in the treated group indicate marked inhibition of thromboxane synthesis, whereas plasma concentrations of 6-oxo-prostaglandin $F_{1\alpha}$ were not significantly lower than in controls. Thus the combination of low-dose aspirin and dipyridamole directed the maternal prostacyclin/thromboxane A_2 balance to the dominance of prostacyclin. The results of various studies suggest that this effect is mainly caused by the low dose of aspirin,[7, 8] which inhibits platelet cyclo-oxygenase in the presystemic (portal) circulation.[12] However, our study design does not permit assessment of the relative contribution of dipyridamole, a phosphodiesterase inhibitor considered to potentiate the action of aspirin by delaying the breakdown of cyclic adenosine monophosphate, by rendering platelets more sensitive to prostacyclin and perhaps by stimulating prostacyclin synthesis.[8]

Our method of selection of patients to be treated led to inclusion of the most severe cases of recurrent fetal growth retardation in the treatment group, whereas the histories of controls were significantly less complicated. Despite this bias in favor of the controls, 61% of control patients had another low birth weight infant, in comparison with only 13% in the treated group. The high recurrence rate in the control group is in agreement with results from other studies.[13] The significantly better outcome in the treatment group cannot be attributed to differences between groups with respect to ethnic background, smoking habits, antenatal care, or duration of gestation at delivery; this strongly suggests that low-dose aspirin and dipyridamole given from 16 to 34 weeks' gestation improves uteroplacental perfusion and fetal growth and prevents placental infarction. It may be of significance that in nine pregnancies a marked reduction in platelet life span was already apparent at 14 weeks' gestation, probably because of increased platelet consumption in the uteroplacental vascular bed caused by formation of thrombi.[2] The observed return to normal values at 34 weeks suggests reversal of the pathologic platelet-vessel wall interaction. The two cases of rather severe fetal growth retardation occurred in patients with evidence of circulating lupus anticoagulant. In these patients suppression of anticoagulant activity with prednisone, in addition to the antiplatelet therapy, might have further improved fetal growth.[11]

Our study supports data from a randomized, but not double-blind, trial reported by Beaufils et al.,[14] who treated 52 patients at high risk for preeclampsia and/or fetal growth retardation with 150 mg aspirin and 300 mg dipyridamole daily from 3 months of gestation until delivery. No severe fetal growth retardation (less than third percentile) occurred in the treated women, compared with seven cases in 50 untreated high-risk control patients.

The low dose of aspirin used in the second and part of the third trimester in combination with dipyridamole did not cause any apparent adverse effects in mother or fetus. Neonatal hemorrhagic complications have been reported after high doses of aspirin (5 to 15 gm) ingested by the mother in the 5 days before delivery.[15] There is no evidence that this occurs with the much lower doses we used, and most patients were delivered at least 1 week after the end of the treatment. The small number of proved cases of in utero constriction of the ductus arteriosus after maternal ingestion of aspirin[16] have also been associated with doses that were much higher than we used.

We realize that a randomized clinical trial with in-

ternal controls would have been the method of choice for our study. For reasons already stated, we had to use external controls. Although such a study will support less strong inferences than a randomized trial, it still has a role in clinical investigation.[17] We feel that the results of our study show sufficient promise to justify further controlled clinical trials to establish the possible benefit of low-dose aspirin, with or without dipyridamole, in the prevention of recurrent idiopathic fetal growth retardation.

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Caffeine-cigarette interaction on fetal growth

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The influence of caffeine and cigarette consumption on fetal growth during pregnancy was studied retrospectively in 913 newborn infants. Analysis of variance was adjusted for length of gestation and sex. A significant caffeine-cigarette interaction was found on birth weight ($F = 85.4$, $p < 0.01$). Among women smoking 15 cigarettes or more per day, birth weight was 206 gm (±57.7 gm) lighter for babies whose mothers consumed 300 mg or more of caffeine per day. Head circumference and length of the newborns were affected only by cigarette smoking. Another caffeine-cigarette interaction was observed on placental weight ($F = 15.0$, $p < 0.01$). Among women who consumed less than 300 mg of caffeine daily, placental weight increased with cigarette consumption. However, it diminished ($p < 0.05$) among women smoking 15 cigarettes or more per day if they consumed 300 mg or more of caffeine daily. This is the first time that such interactions were found to influence birth and placental weights, indicating that the concomitant consumption of caffeine and cigarette constitutes a higher risk for the developing fetus. (Am J Obstet Gynecol 1987;157:1236-40.)

Key words: Caffeine, cigarette smoking, fetus, pregnancy, fetal growth

The caffeine, cigarette, and alcohol consumption of mothers during pregnancy has been the subject of many studies of its effects on fetal growth. Because these substances can easily reach the fetus by placental transfer, it is of prime importance to study the effect of interaction of these substances on fetal growth.

The influence of smoking on birth weight is well known. In fact, birth weight is reported to be inversely proportional to the number of cigarettes smoked by the mother, and this effect is independent on other factors related to the variation in birth weight.[1] The length of the newborn is also reduced when the mother smokes, and some authors have observed a decrease in head circumference.[2] Placental weight is not affected by smoking, but the number of cigarettes smoked has been correlated with hypovascular and atrophic microscopic changes in placental villi[3] and deterioration of placental perfusion type.[4] The reduction in uteroplacental circulation caused by the vasoconstricting effect of nicotine[4] and the relative fetal hypoxia caused by the increase of carbon monoxide concentrations in the fetus[5] are two factors responsible for the decrease in fetal growth observed among babies born to smokers.

On the other hand, maternal alcoholism has been related to a series of physical and neurologic abnormalities of newborns that in 1973 Jones and Smith[6] termed "fetal alcohol syndrome." A significant clinical reduction of newborn weight is observed only at a consumption level higher than 30 ml of absolute alcohol per day.[7]

During pregnancy, the elimination kinetic profile of caffeine was reported to be modified. In fact, Aldridge et al.[8] reported an increase in the half-life of caffeine from 5.3 hours before pregnancy to 18.1 hours during the last two trimesters of pregnancy. These values return to normal soon during the postpartum period. This increase in the half-life of caffeine in the mother coincides with the period during which fetal growth is exponential so that an accumulation of this substance represents a potential risk for the fetus and the placenta, which is responsible for the transfer of nutrients.

To date, no well-controlled study has found that caffeine influences human birth weight. However, it has been demonstrated that a unique dose of 200 mg of caffeine, which is equivalent to two to three cups of coffee, administered to the mother significantly reduces blood flow in placental villi and increases maternal serum epinephrine levels.[9] This effect of caffeine on placental circulation is quite similar to that observed after cigarette consumption.

Smoking is often associated with high caffeine intake, and this has also been confirmed for pregnant women.[10] However, the influence of that concomitant intake on fetal growth was not fully investigated. The association of these two consumption habits could represent a greater risk of disturbing the full development of the fetus. However, to date research has not fully investigated this hypothesis because the effects of caffeine and cigarette consumption on fetal growth have been studied separately.

The effects of caffeine and cigarettes on placental circulation raise the assumption that these substances

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can act in an additive or synergistic way on fetal growth. The main objective of this study was to evaluate the independent effect and the interaction of caffeine and cigarette on weight, head circumference, length of the newborn, placental weight, and placental ratio (placental weight/birth weight × 100).

Methodology

All women delivering at either Centre Hospitalier Chauveau or Hôpital St-François d'Assise in Québec City, Canada, during a 5-month period from February to July 1985 were requested to complete a questionnaire on their caffeine, cigarette, and alcohol consumption habits during pregnancy. Of these 35 women refused to participate while 1014 women entered the study. This low refusal rate of 3% is not thought to bias the results. Standardized verbal and written information was given along with the questionnaires. Data collection was completed by a review of the medical and obstetric records of the mother and baby. The consumption pattern of caffeine, cigarettes, and alcohol of these women during pregnancy has been the subject of a previous publication.[11]

The questionnaire gathered information about the socioeconomic status, participation at prenatal classes, parity, obstetric history, weight before and at the end of pregnancy, height, health problems, and pattern of consumption of caffeine, cigarettes, and alcohol during pregnancy. The reported intake of these substances represents an average across pregnancy.

The caffeine content of coffee, tea, cola, and chocolate used is that reported by the quantitative analysis of Gilbert et al.,[12] and the publication of Graham.[13] The amount of caffeine varied depending on the substance consumed: coffee: expresso = 170 mg/cup of 180 ml, filter or percolator = 110 mg/cup, instant = 75 mg/cup, and decaffeinated = 1 mg/cup; tea: bag = 50 mg/cup and leaves or instant = 30 mg/cup; chocolate = 40 mg/bar of 40 gm; cola: diet or regular = 45 mg/180 ml. These values were used to calculate total caffeine consumption by adding the intake from coffee, tea, cola, and chocolate. Cigarette consumption represents the number of cigarettes smoked per day. Alcohol consumption was calculated in milliliters of absolute alcohol consumed per day. Beer (350 ml), wine (120 ml), appetizer (60 ml), and spirits/digestive (30 ml) contained 15, 13, 13, and 15 ml, respectively, of absolute alcohol per serving.

To study the influence of caffeine, cigarette, and alcohol consumption on fetal growth and exclude other major factors affecting fetal growth, it was necessary to exclude 101 subjects from the results analysis: multiple pregnancies (n = 16), mothers suffering from preeclampsia and chronic hypertension (n = 51), diabetes (n = 8), organic heart disease (n = 5), congenital spherocytosis (n = 1), Val Kecklinhausen (hyperparathyroidia) (n = 1), hypoglycemia (n = 3), babies with major malformations (n = 11), and stillbirths (n = 5).

Table I. Physical characteristics of mothers and newborns

Characteristics	*Mean ± SD*
Mothers	
Weight before pregnancy (kg)	56.3 ± 9.7
Weight at the end of pregnancy (kg)	69.6 ± 10.5
Weight gain (kg)	13.3 ± 4.4
Height (cm)	160.7 ± 6.0
Length of gestation (wk)	39.4 ± 1.6
Placental weight	526.1 ± 121.0
Newborns	
Birth weight (gm)	3352.1 ± 496.3
Head circumference (cm)	34.5 ± 1.5
Length (cm)	51.0 ± 2.3

Statistical analysis was done by means of the statistical analysis system. Level of significance was fixed at 0.05. Analysis of variance for unbalanced groups was done by the introduction of independent variables: caffeine, cigarette, and alcohol consumption and combinations of these variables adjusted for length of gestation and the sex of the babies. The consumption of these substances was divided as follow: alcohol: <2, 2 to 5.9, ≥6 ml of absolute alcohol per day; cigarettes: 0, 1 to 14, ≥15/day; and caffeine <300 and ≥300 mg/day. Dependent variables included birth weight, head circumference, length of newborns, placental weight, and placental ratio. Tukey-Kramer's test for unbalanced groups was used to define the influence of different levels of consumption on fetal growth. Reported means for birth weight, head circumference, and length were adjusted for length of gestation and the sex of the babies, both of which were unevenly distributed in the subgroups studied. Placental weight was adjusted for length of gestation only since it did not vary with the sex of the babies.

Results

After data collection, 101 subjects were excluded since they did not meet the inclusion criteria mentioned. Results are presented for the remaining 913 subjects.

Physical characteristics of babies and mothers are presented in Table I.

Alcohol at doses consumed by women in the present study had no significant effect on the studied parameters. Mean absolute alcohol consumption of this population was 1.5 ml (SD = ±3.2 ml) per day, which represented about three servings of alcoholic beverages per month.

Subjects were then grouped according to caffeine and cigarette consumption. Caffeine consumption was

Table II. Groups according to the caffeine and cigarette consumption of the mothers

Consumption level			*Mean consumption*	
Caffeine (mg/day)	*Cigarettes (No./day)*	*No. of subjects*	*Caffeine (mg/day) mean ± SD*	*Cigarettes (No./day) mean ± SD*
<300	0	536	100.8 ± 83.2	0
<300	1-14	128	119.3 ± 90.6	7.6 ± 3.8
<300	>15	114	135.2 ± 86.5	20.3 ± 5.4
≥300	0	42	407.8 ± 222.7	0
≥300	1-14	24	423.9 ± 116.9	9.3 ± 3.4
≥300	>15	69	442.2 ± 142.7	22.8 ± 6.8

divided into two categories: (1) subjects whose consumption was less than 300 mg/day and (2) those whose daily consumption was greater than 300 mg/day. Among these two categories, women were divided according to their cigarette consumption, either 0, one to 14, or 15 or more per day. For each of these six groups, Table II shows the number of subjects and the mean daily consumption of caffeine and cigarettes.

At the end of pregnancy, smokers represented 36.7% of the study population. Whereas 15.8% of the women who smoked one to 14 cigarettes/day and 37.7% of those who smoked 15 or more cigarettes daily consumed 300 mg or more of caffeine daily, only 7.3% of nonsmokers belonged to the latter category. Among the 135 women (14.8% of the population) whose daily consumption of caffeine rose to 300 mg or more, 68.9% smoked.

Birth weight. Analysis of variance revealed an influence of cigarette ($F = 32.3$, $p < 0.01$) and caffeine-cigarette interaction ($F = 5.8$, $p < 0.01$) on birth weight. For nonsmokers, mean birth weight of babies adjusted for length of gestation and sex of babies was 3441.9 gm (SEM = 16.8 gm). This weight was significantly reduced ($p < 0.05$) for babies born to mothers who smoked; it was 3204.4 gm (SEM = ±39.0 gm) and 3141.7 gm (SEM = ±28.8 gm) for a consumption of one to 14 and 15 or more cigarettes per day, respectively. Fig. 1 presents mean birth weight of babies divided according to maternal caffeine and cigarette consumption. All four smoking subgroups had mean birth weights significantly lower than both nonsmoking subgroups. For nonsmokers and women who smoked one to 14 cigarettes daily, the birth weight was not statistically different with a caffeine consumption less than or greater than 300 mg/day. However, among women smoking 15 cigarettes or more per day, the birth weight of babies of those women who consumed 300 mg or more caffeine daily was significantly ($p < 0.05$) lighter than that of babies whose mothers consumed less caffeine. The difference in birth weight of babies between these two groups was 206 gm (SEM = ±57.7 gm).

When we consider both caffeine and cigarette consumption, the birth weight of babies born to women who smoked 15 cigarettes or more per day was significantly reduced if the mother consumed 300 mg of caffeine or more daily.

Head circumference. Head circumference was influenced by cigarette consumption ($F = 8.2$, $p < 0.01$) but not by caffeine consumption. No caffeine-cigarette interaction was found. Head circumference of babies born to nonsmokers was 34.6 cm (SEM = ±0.06 cm), and it diminished significantly ($p < 0.05$) for babies of mothers who smoked, being 34.3 cm (SEM = ±0.11 cm) and 34.1 cm (SEM = ±0.11 cm) in newborns whose mothers smoked one to 14 and 15 or more cigarettes per day, respectively.

Length. Length of babies was also influenced by cigarette consumption ($F = 17.8$, $p < 0.01$), but again there was no interaction between cigarette smoking and caffeine consumption. Babies born to nonsmokers had a mean length of 51.3 cm (SEM = ±0.09 cm), whereas those born to mothers who smoked one to 14 and 15 or more cigarettes per day were 50.7 cm (SEM = ±0.16 cm) and 50.3 cm (SEM = ±0.15 cm) long, respectively.

Placental weight. Placental and birth weights underwent a caffeine-cigarette interaction ($F = 8.9$, $p < 0.01$). There was no significant difference between placental weight of nonsmokers and placental weight of the two other groups of smokers.

However, when we compared this weight between smokers groups according to their caffeine consumption, a dose-related interaction was seen. Fig. 2 shows mean placental weight adjusted for length of gestation for each consumption group. Among women who consumed less than 300 mg of caffeine daily, placental weight was significantly higher for those who smoked 15 cigarettes or more per day (mean ± SEM = 566.6 ± 12.5 gm) as opposed to nonsmokers (mean ± SEM = 517.5 ± 4.8 gm) ($p < 0.05$) and smokers of one to 14 cigarettes per day (mean ± SEM = 521.2 ± 10.4 gm).

Among women who smoked 15 cigarettes or more per day, placental weight was significantly reduced for those who consumed 300 mg or more of caffeine daily compared with those whose caffeine consumption was

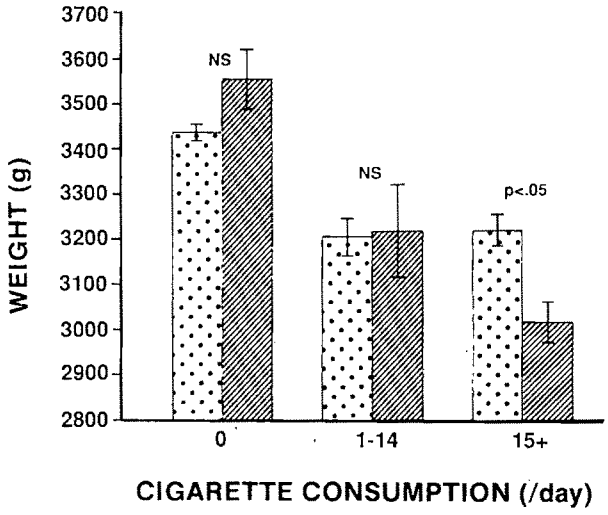


Fig. 1. Effect of caffeine-cigarette interaction on birth weight. Means (±SEM) are adjusted for the length of gestation and sex of newborns. (▩ = Caffeine consumption <300 mg/day; ▨ = caffeine consumption ≥300 mg/day.)

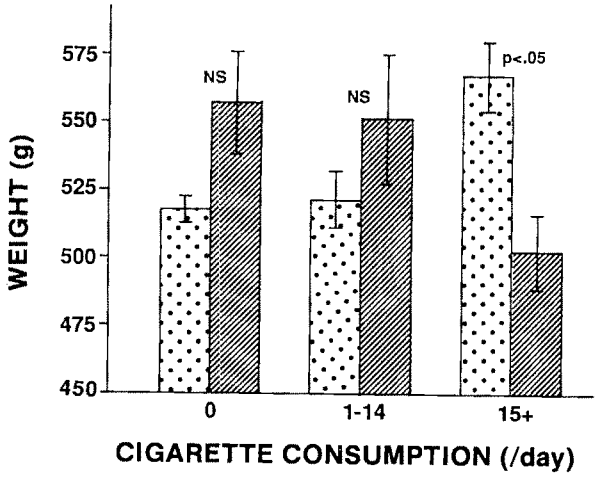


Fig. 2. Effect of caffeine-cigarette interaction on placental weight. Means (±SEM) are adjusted for the length of gestation. (□ = Caffeine consumption <300 mg/day; ▨ = caffeine consumption ≥300 mg/day.)

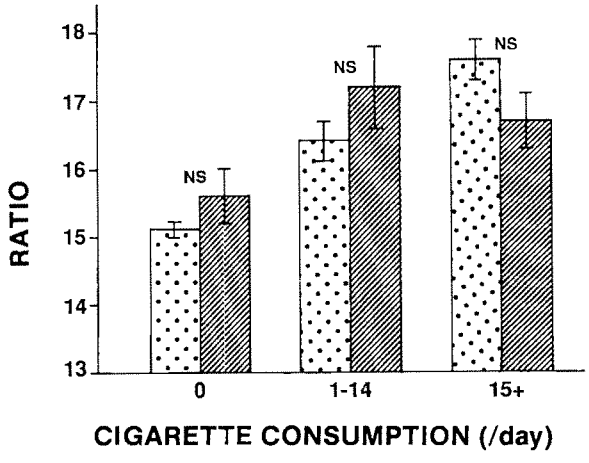


Fig. 3. Effect of caffeine-cigarette interaction on placental ratio. Means (±SEM) are adjusted for the length of gestation and sex of newborns. (▩ = Caffeine consumption <300 mg/day; ▨ = caffeine consumption ≥300 mg/day.)

less than 300 mg/day ($p < 0.05$); placental weight was 502.3 gm (SEM = ±13.8 gm) and 566.6 gm (SEM = ±12.5 gm), respectively.

Placental ratio. The ratio of placental weight to birth weight was affected by cigarette consumption ($F = 12.8$, $p < 0.01$) and underwent a caffeine-cigarette interaction ($F = 3.3$, $p < 0.05$) (Fig. 3). Placental ratio increased with cigarette consumption, going from 15.1 (SEM = ±0.12) for nonsmokers to 16.5 (SEM = ±0.26) and 17.3 (SEM = ±0.26) for women who smoked one to 14 and 15 or more cigarettes per day, respectively. This reflects the increase in placental weight and the decrease in birth weight observed with an increase in the number of cigarettes smoked.

Considering both caffeine and cigarette consumption, the placental ratio increased significantly ($p < 0.05$) for every consumption level of cigarettes for women who consumed less than 300 mg of caffeine/day. However, for women who consumed 300 mg or more of caffeine daily, there was no significant difference in placental ratio according to cigarette consumption. Among women who smoked 15 or more cigarettes a day, the placental ratio was not statistically different with a caffeine consumption of less than or greater than 300 mg/day, since both birth and placental weights diminished when caffeine consumption was 300 mg or higher.

Comment

This study confirms that cigarette consumption during pregnancy reduces weight, head circumference, and length of the newborn infant. Placental weight is not affected by smoking. These results are consistent with those of other studies.[3] Since placental weight is stable and birth weight diminishes with cigarette consumption, an increase in the placental ratio results. This effect was also observed by Wingerd et al.[14] Wilson[15] suggested that smoking causes chronic fetal hypoxia, which results in a reduction of fetal growth, and that the relative placental hypertrophy is compensatory. Even if cigarette consumption does not seem to affect placental growth negatively in terms of weight, it has been shown that it produces a reduction of placental blood flow[3, 4] and that it increases morphologic anomalies of the placenta.[16] Many changes in the ultrastructure of the placenta of smokers have been observed[17] and might induce a decrease in metabolism and diffusion through the placental barrier.

Linn et al.[18] concluded that independent of cigarette consumption, the incidence of low birth weight was not affected by maternal consumption of coffee or tea. Van den Berg[10] observed that the percentage of babies weighing 2500 gm or less was 9% in smokers who consumed more than six cups of coffee per day. Because 4.4% of the nonsmokers consumed the same quantity of coffee daily and because the latter percentage was equal to that observed in the total cohort, he attributed the difference entirely to cigarette consumption without considering that this effect might be caused by the association between caffeine and cigarettes. These authors have calculated caffeine consumption in the number of cups of coffee per day without taking into account the eventual consumption of decaffeinated coffee. In addition, tea consumption was transformed into cups of coffee without correcting for its smaller caffeine content. Other sources of caffeine, such as cola and chocolate, were not considered.

The results of our study show that caffeine consumption during pregnancy has a negative effect on the birth weight of babies born to smokers of 15 or more cigarettes per day. Doses of 300 mg or more of caffeine per day in these women accentuate the decrease caused by smoking. It is the first time that such an effect has been demonstrated. Moreover, the interaction between caffeine consumption and smoking affects placental weight in a particular manner. For women who consume less than 300 mg of caffeine/day, the placenta could compensate for the effect of cigarettes by real hypertrophy. Nevertheless, for women who smoke 15 or more cigarettes a day and consume 300 or more mg of caffeine daily, placental weight is reduced compared with women who consume less caffeine.

To explain the underlying mechanisms of the caffeine-cigarette interaction on birth and placental weights, further microscopic and ultrastructural studies are required since morphometric measures alone do not permit complete definition of the functional capacity of the placenta.

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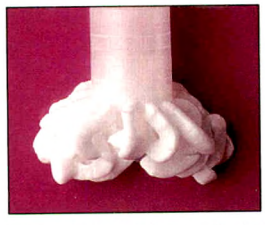


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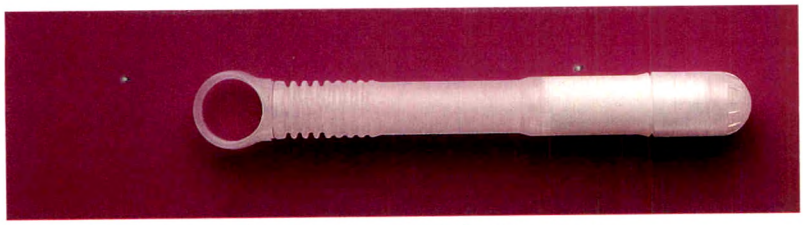
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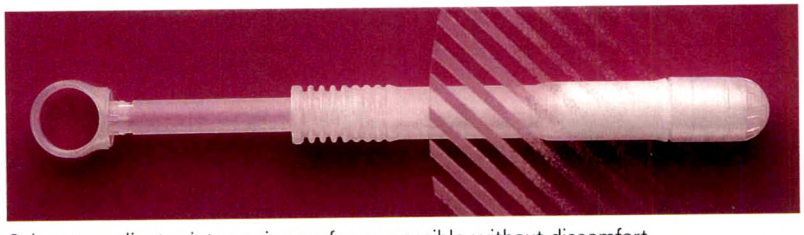
Femstat® Prefill™

(butoconazole nitrate)

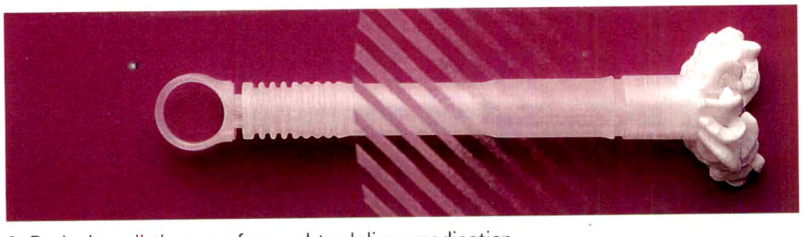
As simple as 1-2-3...



1. Peel back protective foil to remove applicator. Do not attempt to remove special dispersion cap on tip of applicator.

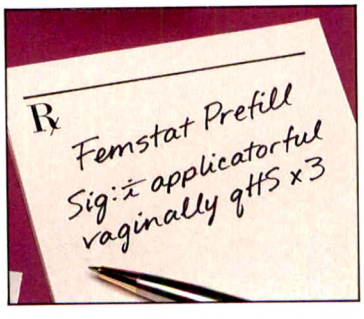


2. Insert applicator into vagina as far as possible without discomfort. Pull back on ring.



3. Push ring all the way forward to deliver medication. Withdraw applicator and discard.





SYNTEX

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BRIEF SUMMARY

INDICATIONS:
FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS:
FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS:
General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

February 1987

The effect of smoking on placental and fetal zinc status

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Previous studies have reported a cadmium/zinc interaction in cadmium-exposed pregnant animals that results in (1) increased placental cadmium levels, (2) increased placental zinc levels, and (3) decreased placental zinc transport. This study was carried out to determine whether zinc status would be affected in pregnant women exposed to cadmium through cigarette smoke. Atomic absorption spectroscopy was used to determine the levels of cadmium and zinc; 65 pregnant women who smoke and 84 who do not smoke were studied. Our data reveal that increased cadmium levels in pregnant women as the result of smoking increase placental zinc levels and decrease cord red blood cell zinc levels. Significantly higher levels of both cadmium and zinc were found in the placentas of pregnant women who smoke; moreover, stepwise multiple regression showed that maternal whole blood cadmium levels predicted placental zinc levels. In regard to cord blood, a significant 9% decrease in the red blood cell zinc level was observed in infants of mothers who smoke and this decrease was correlated with smoking activity, as evaluated by measuring plasma levels of thiocyanate. Also cord red blood cell zinc levels were found to correlate with placental zinc levels in nonsmokers but not in smokers. Overall, our data show that a cadmium/zinc interaction does take place in the maternal-fetal-placental unit of pregnant women who smoke and results in less favorable zinc status in the infants. (Am J Obstet Gynecol 1987;157:1241-6.)

Key words: Smoking, placental zinc, fetal zinc

Zinc nutritional status has a profound influence on the biochemistry and toxicology of cadmium. One reason for this is that cadmium and zinc often act antagonistically in biologic systems; for example, cadmium acts as an antagonist to zinc in zinc-requiring metalloenzymes, for instance, carbonic anhydrase,[1] alkaline phosphates,[2] and others.[3] Another reason is that metallothionein, which is produced in response to cadmium exposure, binds both cadmium and zinc; thus, if zinc levels are marginally adequate, zinc status may be aggravated by the binding and storage of zinc by cadmium-induced metallothionein. Finally, an inadequate zinc nutritional status can influence the absorption and tissue uptake of cadmium, thereby increasing the probability of a toxic effect due to cadmium.[4]

Pregnant women may be particularly susceptible to this cadmium/zinc interaction. They are susceptible because their zinc intake is generally substantially lower than the Recommended Daily Allowance of zinc during pregnancy[5] and significant associations have been reported between fetomaternal complications and plasma zinc levels.[5] Even more vulnerable, however, is the pregnant woman who smokes cigarettes. The average cigarette contains as much as 1 μg of cadmium, and heavy smokers may have an intake of 20 μg/day or more as the result of smoking, in addition to the average daily intake of 50 μg/day from dietary sources. Thus decreased zinc nutritional status and increased cadmium intake may promote a cadmium/zinc interaction in pregnant women who smoke.

The magnitude of this cadmium/zinc interaction in pregnant women who smoke is of course important since it could influence fetal growth and development. Low zinc concentrations in maternal blood and cord blood have been related to abnormal events in parturition, intrauterine growth retardation, and increased incidences of congenital malformations in humans.[5] Moreover, previous research on pregnant animals has clearly shown that this interaction occurs between cadmium and zinc and can result in the fetus receiving less zinc.[5]

Accordingly, we investigated the zinc status and cadmium levels of pregnant women who smoke. Our primary purpose was to determine whether the cadmium levels were related to zinc status in the mother and fetus. We report herein the results of our investigation.

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Table I. Characteristics of the maternal study population

Characteristic	Smokers (n = 65)		Nonsmokers (n = 84)	
	n	%	n	%
Maternal age (yr)	24 ± 4		25 ± 4	
Race				
Black	19	29	34	40
White	45	69	44	52*
Other	1	2	6	7
Parity				
Nulliparous	12	18	16	19
Multiparous	53	82	68	81
Delivery method				
Vaginal	35	54	40	48
Cesarean section				
Primary	9	14	11	13
Repeat	21	32	33	39
Complications				
None	43	66	53	63
Hypertension	1	2	1	1
Rh sensitization	0	0	1	1
Preeclampsia	2	3	2	2
Diabetes				
Class A	1	2	4	5
Classes B to F and R	2	3	1	1
Mild anemia	4	6	3	4
Weight				
>200 pounds	5	8	12	14
<100 pounds	0	0	0	0
Precipitate labor	5	8	7	8
Long labor	2	3	3	4
Prolonged second stage	1	2	4	5
Premature rupture of membranes	0	0	0	0

*$p < 0.05$.

Table II. Characteristics of the infant study population

Characteristic	Smoking mother (n = 65)		Nonsmoking mother (n = 84)	
	n	%	n	%
Birth weight (gm)	3161 ± 597		3515 ± 533*	
Gestation (wk)	38.8 ± 2		39.3 ± 2	
Apgar score <7				
1 min	11	17	11	13
5 min	2	3	1	1
Sex				
Male	37	57	43	51
Female	28	43	41	49
Complications				
Small for dates	5	8	0	0*
Large for dates	10	15	32	39
Premature	4	6	5	6
Postterm	1	2	5	6

*$p < 0.05$.

Methods and material

Patients and collection of samples. Sixty-five pregnant women who smoke and 84 who do not smoke and their fetuses were included in this study. The only selection criteria were that the mothers resided in or very near Cleveland, Ohio, and were delivered of their infants at Cleveland Metropolitan General Hospital. They were not selected with regard to prenatal or intrapartum medical problems, although in depth clinical information was recorded. Informed consent for this study was obtained from the subjects in the appropriate manner.

Samples of blood were obtained from the mothers within an hour of delivery and from the umbilical cord immediately after delivery. Samples of 10 ml were procured in separate heparinized polypropylene syringes from the antecubital vein of the mother and umbilical cord vein of the infant. Samples of placental tissue were obtained after several cotyledons had been perfused with 2 L of cold 0.9% saline solution to remove excess blood, and the membranes and blood vessels were removed. Each sample was then stored frozen until analyzed for levels of whole blood cadmium (maternal blood only), placental cadmium, placental zinc, plasma zinc, and red blood cell zinc. Maternal plasma was also analyzed for thiocyanate, which was used in conjunction with questionnaires for assessing smoking behavior.

Zinc intake in a random subgroup of the population (29 smokers, 37 nonsmokers) was assessed by diet history at term. The diet history included a 7-day food frequency assessment that was verified for accuracy by a 24-hour recall. Food sources known to be the major contributors of zinc in the diet (for example, meat, seafood, eggs, dairy products, dried beans and peas, and whole grain breads and cereals) were quantified and an estimate of weekly zinc intake in milligrams was calculated.

Determination of cadmium, zinc, and thiocyanate levels. The samples of blood and placental tissue were prepared for analysis of cadmium by means of acid digestion. The digestion mixture was concentrated Ultrex (J. T. Baker, Phillipsburg, New Jersey) nitric acid and hydrogen peroxide. The details of this procedure were previously described by us.[7]

An atomic absorption spectrophotometer (Model 560, Perkin Elmer Corp., Norwalk, Connecticut) equipped with a deuterium background corrector and a graphite furnace (Model HGA-2200, Perkin Elmer Corp.) was used for the cadmium analyses.

In contrast to the analysis of cadmium, flame atomic absorption analysis was used for the analysis of zinc. Red blood cell and plasma zinc was analyzed after a 1:4 dilution with deionized water. Glycerol was added

Table III. Indices of smoking

	Smokers (N = 65) (mean ± SD)	*Nonsmokers (N = 84) (mean ± SD)*	*Significance*
Thiocyanate (μmol/L)	107 ± 40	30 ± 14	$p < 0.05$
Maternal whole blood cadmium (ng/gm)	1.33 ± 0.8	0.80 ± 0.4	$p < 0.05$
Placental cadmium (ng/gm)	12.0 ± 7.5	8.1 ± 5.0	$p < 0.05$

Table IV. Indices of zinc status

	Smokers (N = 65)	*Nonsmokers (N = 84)*	*Significance*
Mother			
Serum alkaline phosphatase (units/100 ml)	6.3 ± 2.8	6.0 ± 2.3	NS
Plasma zinc (μg/100 ml)	57.5 ± 9.7	57.0 ± 10.0	NS
Red blood cell zinc (μg/100 ml)	1232 ± 169	1218 ± 150	NS
Placenta			
Zinc (ng/gm)	12.1 ± 2.7	11.1 ± 2.8	$p < 0.05$
Alkaline phosphatase (units/100 ml)	9.0 ± 4.5	7.4 ± 3.6	$p < 0.05$
Infant cord blood			
Alkaline phosphatase (units/100 ml)	9.4 ± 2.8	10.0 ± 3.1	NS
Plasma zinc (μg/100 ml)	81.1 ± 14.5	83.2 ± 15.0	NS
Red blood cell zinc (ng/gm)	230 ± 55	250 ± 60	$p < 0.05$

to the blank and standards to approximately match the viscosity of the diluted plasma. Placental zinc was analyzed after acid digestion as described under cadmium analysis.

Plasma thiocyanate, a byproduct of the metabolism of cyanide in cigarette smoke, was measured by means of the procedure described by Pettigrew and Fell.[8] Patients with thiocyanate values ≤59 μmol/L were defined as nonsmokers and those with values of ≥60 μmol/L were defined as smokers in the few cases where information obtained from the clinical record was not consistent with the thiocyanate data. Passive smokers were considered nonsmokers unless the thiocyanate values were ≥60. Maternal, fetal, and placental alkaline phosphatase levels were quantitated with the method of Doellgast and Meis.[9]

Data analysis. Statistical analyses were performed with the BMDP UCLA statistical software package and a DEC Vax 11/750 computer (Digital Equipment Corporation, Maynard, Massachusetts) with a Unix operating system. Data analysis consisted of descriptive statistics, *t* tests, and simple correlations to produce an overview of significant differences between the groups and interrelationships between the various biochemical indices. The χ^2 analysis was used to test for differences in clinical parameters between the two groups. Then stepwise multiple regression techniques were used to examine the interactions between cadmium exposure and zinc status while controlling for statistically confounding variables such as maternal age, parity, race, delivery method, vitamin intake, and diabetes. In some cases (placental cadmium, placental zinc, thiocyanate), the variables were not normally distributed. In these cases, log transformations were used. Statistical significance was the same regardless of whether a log transformation was used. Significance was accepted at $p < 0.05$.

Results

The characteristics of the mothers and infants in this study are shown in Tables I and II, respectively. The smokers and nonsmokers are classified separately. Table I shows that the maternal population groups had similar characteristics except for the proportion of white patients who smoked; a significantly higher proportion of the white patients were smokers. Table II shows that the mean birth weight of the infants of mothers who smoked was less than that of the infants of mothers who did not smoke ($t = 4.7$; $p < 0.0001$). Also more small-for-dates infants were born to mothers who smoked than to mothers who did not smoke. These findings are in agreement with previous studies concerning pregnant women who smoke.

The blood levels of thiocyanate of the two study populations are shown in Table III. The mean levels in the mothers who smoked were significantly higher than those in the nonsmokers.

Illustrated in Fig. 1 and Table III are the relative levels of cadmium found in maternal blood and in the placentas of smokers and nonsmokers. The percent increase in cadmium in the whole blood of smokers was 64%; the percent increase in placental cadmium was 45%. In both smokers and nonsmokers more cadmium was found in the placentas than in the blood. These

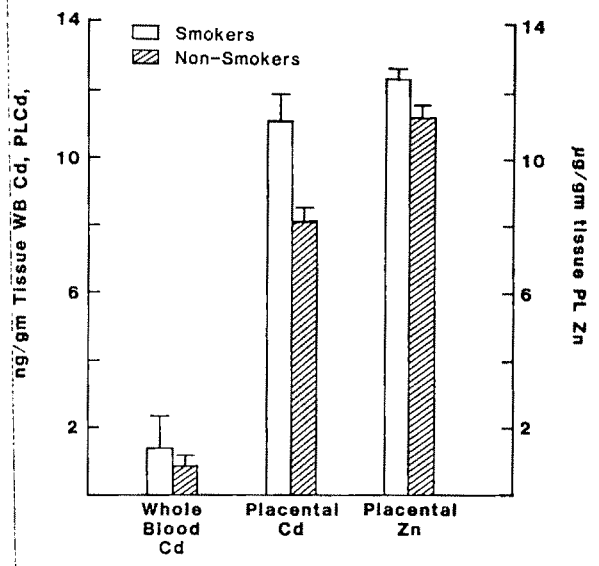


Fig. 1. The effect of smoking on whole blood cadmium, placental cadmium, and placental zinc levels.

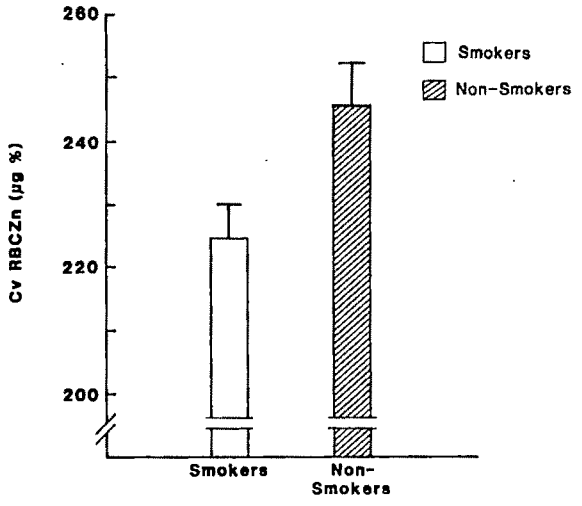


Fig. 2. Cord vein red blood cell zinc levels in smokers and nonsmokers.

findings are in agreement with previous studies concerning cadmium exposure in pregnant women who smoke.[6, 10-12]

The zinc status of smokers and nonsmokers and that of their infants (as assessed by measurements of plasma and red blood cell zinc levels and alkaline phosphatase activity) is compared in Table IV. No significant differences were observed in the mothers. Moreover, diet histories verified that there was no difference in zinc intake between pregnant women who smoked and those who did not. The average daily intake of zinc was 10.4 mg. A major finding of the study was the 9% decrease in the red blood cell zinc level in the cord blood of infants of mothers who smoke (Fig. 2); this difference is statistically significant.

A comparison of the zinc status of the mothers and infants shows that the infants had plasma zinc and alkaline phosphatase levels that were approximately one third higher than those of their mothers; on the other hand, the red blood cell zinc levels of the infants were approximately five times lower than those observed in the mothers.

Comparisons of the levels of zinc in the placentas of smokers and nonsmokers are shown in Table IV and Fig. 1. Significantly higher levels of zinc were found in the placentas of pregnant women who smoked. The significantly higher levels of placental zinc correlated with significantly higher levels of alkaline phosphatase in the placenta.

In smokers, the significant predictor of both placental zinc and placental cadmium by stepwise multiple regression was maternal whole blood cadmium. In nonsmokers, the maternal whole blood cadmium level predicted the placental cadmium level but not the placental zinc level. These data are summarized in Table V, which shows the F value, degrees of freedom, and p value for each analysis.

Since a 9% decrease in the red blood cell Zn level was observed in the cords of infants of mothers who smoke, the relationships between the biochemical variables and cord vein red blood cell zinc levels were examined. Significant predictors of the cord vein red blood cell zinc level, as assessed by stepwise multiple regression, were different in the smoking and nonsmoking groups. In smokers, thiocyanate was the strongest predictor of the cord vein red blood cell zinc level. The simple correlation showed that the relationship was negative, that is, the more thiocyanate in maternal plasma, the less zinc in the red blood cells of the infant. In nonsmokers, placental zinc was the strongest predictor of cord vein red blood cell zinc. The simple correlation showed that the relationship was positive, that is, the more placental zinc, the more cord vein red blood cell zinc. These data are summarized in Table VI, which shows the F value, degrees of freedom, p value, and r value for each analysis.

Comment

The principal purpose of this study was to determine whether the increased levels of cadmium found in pregnant women who smoke affects the distribution of zinc in the maternal-fetal-placental unit. Our data show that increased cadmium levels as the result of smoking influence the levels of zinc in the placenta and cord blood and that this effect of cadmium is significant even though the absolute increase in cadmium as the result of smoking is small.

To determine the effect of increased cadmium ex-

Table V. Significant relationships between maternal whole blood cadmium and placental cadmium or zinc in smokers and nonsmokers obtained with stepwise multiple regression

	Smokers	*Nonsmokers*
Placental cadmium	$F[2,59] = 4.6$; $p < 0.05$	$F[1,83] = 4.7$; $p < 0.05$
Placental zinc	$F[1,60] = 15.3$; $p < 0.001$	$F[2,82] = 3.0$; NS

Table VI. Predictors and univariate correlation coefficients of cord vein red blood cell zinc in smokers and nonsmokers

	Smokers	*Nonsmokers*
Predictor	Thiocyanate	Placental zinc
Stepwise multiple regression	$F[2,58] = 4.14$; $p < 0.05$	$F[2,82] = 5.95$; $p < 0.025$
Univariate correlation coefficient	$r = 0.21$; $p < 0.05$	$r = 0.21$; $p < 0.05$

posure on zinc status in pregnant women who smoke, we first verified that our study population of pregnant smokers and nonsmokers (with similar clinical characteristics) had similar levels of zinc intake. This was done by comparing diet histories and several indices of zinc status of the two groups. Our data showed that there was no difference in zinc intake between the smokers and the nonsmokers and that there was no difference in plasma zinc and red blood cell zinc levels. The data also showed that zinc intake of both groups was about 50% below the Recommended Daily Allowance for zinc during pregnancy, as reported by other investigators studying healthy pregnant patients.[5] However, since both groups had decreased zinc intake and were similar in all other respects, a comparison could be made in regard to the effect of cadmium exposure on zinc status.

The cadmium exposure of our study population as the result of smoking was similar to that reported by us[6] and by others.[10-12] We observed a 64% increase in whole blood cadmium and a 45% increase in placental cadmium in the present study. The percent increase in placental cadmium reported to date has ranged from 26[10] to 400.[11, 12] The values differ, of course, depending on the study population and on the analytic techniques used. However, all the studies agree that there is a substantial increase in cadmium in the placentas of pregnant women who smoke.

Increased exposure to cadmium and decreased zinc intake, as found in our pregnant smokers, are conditions that favor an effect of smoking on zinc status. Data supporting this are based on studies involving animals. For example, studies by Webb and Samarawickrama[13] on rats show that maternal cadmium exposure causes fetal zinc deficiency and that this is one mechanism of the teratogenic effects of cadmium. By giving cadmium to pregnant rats these workers were able to show a dose-related decrease in fetal uptake of zinc. Additional evidence that cadmium can induce fetal zinc deficiency was reported by Daston.[14] This author showed that coadministration of zinc almost eliminated the severe lesions in the fetal lung when the pregnant rat was given cadmium. Although the levels of cadmium in these studies are much higher than we observed in our study population, the threshold for such effects has not been established for animals or humans. Moreover, our data suggest that a cadmium/zinc interaction takes place in the maternal-fetal-placental unit of pregnant women who smoke.

One aspect of our data that suggests a cadmium/zinc interaction and an effect of smoking on zinc status is the 10% increase in the level of zinc in the placentas of pregnant women who smoke. This is suggestive because it is likely that the increased levels of cadmium as the result of smoking induce the production of metallothionein, a low molecular weight protein that binds both cadmium and zinc. With the increased production of metallothionein there is an increased binding of cadmium and zinc and consequently a sequestering of these metals in the placenta. Although this has not been specifically studied in the placenta, autopsy studies of the kidney have shown that zinc levels increase in the kidney cortex with increasing concentrations of cadmium, and this increase is almost equimolar to the increase in cadmium.[15] Support for this interpretation is also provided by our statistical analysis, which shows that in smokers maternal whole blood cadmium levels are predictive not only of the placental cadmium levels but also of the placental zinc levels.

Another aspect of our data that suggests an effect of smoking on zinc status is the 9% decrease in red blood cell zinc observed in the cords of infants of mothers who smoke. This decrease was found to correlate with the levels of thiocyanate (an index of the number of cigarettes smoked) in maternal blood. Thus the greater the number of cigarettes smoked, the lower the level

of red blood cell zinc observed in cord blood. An explanation for this, which is supported by our data, is that zinc bound to metallothionein is accumulating in the placenta and that this binding may reduce the amount of zinc available to the fetus. Another possibility is that less zinc is being transported by the placenta to the fetus; this has been shown to occur in cadmium-injected pregnant rats.[16] Regardless of the explanation, however, the data show that a cadmium/zinc interaction occurring in pregnant women who smoke may be harmful to the fetus since the decrease in cord red blood cell zinc is related to decreased birth weight in the infants of smokers.[17]

Finally, additional evidence of the effect of smoking on fetal zinc status was noted in our data when the relationship between placental zinc levels and cord vein red blood cell zinc levels was examined. The data show a positive correlation between placental zinc and cord vein red blood cell zinc in nonsmokers; in smokers, however, this relationship was not found. The cadmium/zinc interaction, that is, the altered metabolism of zinc brought about by increased cadmium intake due to cigarette smoking, may explain the lack of this correlation in smokers.

The effect of smoking on zinc status in the placenta and fetus may have no health significance if the availability of zinc is above the real requirement. On the other hand, this interaction may be detrimental to the fetus where zinc status is marginal. Additional studies are needed to clarify this point and to determine if zinc supplementation can counteract these changes in tissue levels (as it does in animals). Nevertheless, our studies show that there is an adverse effect of smoking on zinc status in the placenta and fetus, that is, a cadmium/zinc interaction does take place in the maternal-fetal-placental unit of pregnant women who smoke. This finding provides one additional reason for discouraging pregnant women from smoking.

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The relationship between cadmium, zinc, and birth weight in pregnant women who smoke

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It is universally accepted that smoking during pregnancy results in decreased infant birth weight. However, the mechanism for decreased birth weight is not completely understood. This study tested the hypothesis that the cadmium/zinc interaction in the maternal-fetal-placental unit of the mother who smokes could be related to birth weight. Thiocyanate was used as the index of smoking status and atomic absorption spectroscopy was used to determine trace elements. Results show that cord vein red blood cell zinc and maternal whole blood cadmium levels are significant predictors of infant birth weight when variance that is due to clinical fctors and thiocyanate is controlled with stepwise multiple regression techniques (n = 202). Bivariate correlation techniques showed that the factors affecting birth weight were different in the smoking and nonsmoking groups. For example, in nonsmokers (n = 125), the cord vein red blood cell zinc level was positively related to birth weight. In smokers (n = 77), maternal whole blood cadmium, placental cadmium, and placental zinc levels were negatively related to birth weight; the ratio of placental zinc to placental cadmium and the cord vein red blood cell zinc level were positively related to birth weight. The results suggest that increased maternal cadmium and decreased cord vein red blood cell zinc levels in infants of smokers may be significant clinically since increased maternal whole blood cadmium and decreased cord vein red blood cell zinc levels are both significantly related to decreased birth weight.
(AM J OBSTET GYNECOL 1987;157:1247-51.)

Key words: Smoking, cadmium, zinc, birth weight

Smoking during pregnancy results in infants with lowered birth weights.[1] The traditional explanation for the smaller infants has been the hypoxia from carbon monoxide and constriction of the uterine blood vessels from nicotine.[2, 3] However, there are many other compounds in cigarette smoke that may be harmful. One of these is the toxic element cadmium. Each cigarette smoked contributes approximately 1 μg of cadmium to the body's burden of cadmium.[4]

An explanation for the smaller infants is suggested by the interaction between cadmium and zinc in the placenta of mothers who smoke.[5] Zinc is required for many aspects of fetal growth and development.[6] We have recently reported that the cadmium in cigarette smoke may decrease the transfer of zinc across the human placenta.[5] Pregnant women who smoke have higher levels of cadmium and zinc in the placenta and their infants have significantly lower levels of zinc in the red blood cells.[5] Furthermore, cadmium may have adverse effects because it competes with zinc in zinc-requiring metalloenzymes.

The purpose of this study was to test the hypothesis that the cadmium and the altered zinc metabolism reported in the maternal-fetal-placental unit of the mother who smokes could be related to lowered infant birth weight.

Methods and material

Patients and collection of samples. Seventy-seven pregnant women who smoke and 125 who do not and their fetuses were included in this study. The only selection criteria for this survey were that the mothers resided in or very near Cleveland, Ohio, and were delivered of their infants at Cleveland Metropolitan General Hospital. They were not selected with regard to prenatal or intrapartum medical problems, although in depth clinical information was recorded. Informed consent for this study was obtained from the patients in the appropriate manner. The clinical characteristics of most of these patients and the outcomes of their pregnancies have been described previously.[5]

Samples of blood were obtained from the mothers

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Table I. Predictor variables used for bivariate correlation analysis with birth weight

Variables	*Index*
Confounding variables (clinical)	
Maternal age*	
Gravidity*	
Parity*	
Gestational age*	
Sex	
Race*	
Maternal red blood cell count*	
Maternal hemoglobin	
Hematocrit	
Cord vein hemoglobin	
Predictor variables (biochemical)	
Thiocyanate*	Smoking exposure
Maternal whole blood cadmium*	Smoking exposure
Cord vein red blood cell zinc*	Fetal zinc status
Cord vein plasma zinc	Fetal zinc status
Maternal red blood cell zinc	Maternal zinc status
Maternal plasma zinc	Maternal zinc status
Maternal alkaline phosphatase	Maternal zinc status
Heat-stable alkaline phosphatase	Placental zinc status
Placental cadmium*	Zinc-cadmium interaction
Placental zinc†	Zinc-cadmium interaction
Placental zinc/cadmium ratio*	Zinc-cadmium interaction

*Significant independent variables subsequently used for stepwise multiple regression analyses.
†Not significant but used because the ratio of placental zinc to placental cadmium was significant.

within an hour of delivery and from the umbilical cord immediately after delivery. Placental samples were obtained immediately after delivery. Samples were analyzed for determination of whole blood cadmium (maternal blood only), placental cadmium, placental zinc, plasma zinc, and red blood cell zinc. The procedures for sample collection, analysis of the elements by atomic absorption spectrometry, and determination of smoking status with thiocyanate have been reported previously.[5] Smoking status was determined by analysis of plasma thiocyanate, by the clinical record, and by questionnaire.

Data analysis. Statistical analyses were performed with the BMDP statistical software package[7] and a Dec Vax 11/750 computer with a Unix operating system. Descriptive statistics, Student's *t* test, and simple correlations were used to get an overview of distributional properties, significant bivariate differences between the groups (smokers and nonsmokers), and interrelationships between the various clinical and biochemical indices.

Stepwise multiple regression techniques were then used to examine the dependency of birth weight on cadmium exposure and zinc status, after adjusting for significant potential confounding clinical variables such as maternal age, parity, gravidity, race, and maternal red blood cell count. Variables used for both bivariate analyses and stepwise multiple regression analyses are listed in Table I. The sample size was adequate for study of the final 12 variables by stepwise multiple regression.

Placental zinc was not statistically significant with bivariate analysis but was used for stepwise multiple regression analyses because the placental zinc/placental cadmium ratio was significant. This ratio is defined as the ratio of the concentration of placental zinc divided by the concentration of placental cadmium multiplied by 1000.

For variables not normally distributed (placental cadmium, placental zinc, and thiocyanate), log transformations were used where appropriate. Statistical significance was the same regardless of whether a transformed variable was used. Significance was accepted at $p < 0.05$.

The variables selected for multivariate analyses were those yielding significant bivariate associations with the outcome variables of interest (Table I). These analyses were done first on both smokers and nonsmokers together, in order to produce a large range of birth weights and thiocyanate values, and then were also carried out on smokers and nonsmokers separately.

Results

Results of the biochemical determinations have been reported previously.[5] Significant relationships were found between the altered disposition of zinc and cadmium due to smoking and the decreased birth weights in infants of smokers (3143 ± 554 versus 3534 ± 555 gm in nonsmokers). The results of the biochemical variables have been reported previously.[5] Table II shows the results of the multiple regression analyses with birth

Table II. Variability (percent) in birth weight explained by clinical and biochemical variables by use of stepwise multiple regression analyses, with clinical variables forced into the equation before the biochemical variables

Variable	*% of Variance accounted for*		
	Smokers (N = 77)	*Nonsmokers (N = 125)*	*Smokers and nonsmokers (N = 202)*
Confounding variables (clinical)			
Gestational age	11.6*	10.3†	11.5†
Maternal red blood cell count	6.4*	NS	3.4†
Gravidity	3.6†	NS	1.7‡
Maternal age	3.3§	NS	NS
Race	NS	3.05‡	NS
Parity	NS	1.91‡	NS
Predictor variable (biochemical)			
Thiocyanate	5.8†	4.4†	12.2†
Maternal whole blood cadmium	8.5‡	NS	2.5†
Cord vein red blood cell zinc	1.7‖	3.3*	1.5†

NS = Not significant (variance <1%).
*$p < 0.005$.
†$p < 0.001$.
‡$p < 0.05$.
§$p < 0.025$.
‖$p < 0.10$.

Table III. Significant bivariate correlations with infant birth weight

Variable	*Smokers (N = 77)*	*Nonsmokers (N = 125)*	*Smokers and nonsmokers (N = 202)*
Confounding variable (clinical)			
Gestational age	0.34	0.32	0.34
Race	NS*	−0.19†	NS
Maternal red blood cell count	0.27	NS	.23
Predictor variable (biochemical)			
Thiocyanate	−0.33	NS	−0.39
Maternal whole blood cadmium	−0.35	NS	−0.31
Placental cadmium	NS	NS	−0.19
Placental zinc/cadmium ratio	0.31	NS	NS
Cord vein red blood cell zinc	0.33	0.23	0.32

*Not significant ($p < 0.05$).
†Nonwhite infants of smoking mothers weighed slightly less than white infants.

weight as the dependent variable. Only predictors reaching significance at the 5% level for at least one group are shown. After controlling for the clinical variables, thiocyanate (that is whether or not the mother smoked) accounted for a large percentage of the variance ($F[7,193] = 33.1$, $p < 0.001$) that remained. After thiocyanate, the strongest predictors of birth weight were maternal whole blood cadmium ($F[8,192] = 7.1$, $p < 0.001$) and cord vein red blood cell zinc ($F[9,191] = 4.2$, $p < 0.001$). All of the variables (including those that were not significant) accounted for more than 30% of the variance in birth weight that was found.

With the use of simple correlations, it could be shown that there were different relationships between the biochemical variables and birth weight, depending on smoking status. This is the major finding of the study. Table III shows the significant simple correlations found with birth weight. It can be seen that maternal whole blood cadmium and thiocyanate are negatively related to birth weight in smokers. Furthermore, cord vein red blood cell zinc and the ratio of placental zinc to placental cadmium are positively related to birth weight, that is, the more zinc in the cord vein red blood cells or the more zinc than cadmium in the placenta, the greater the birth weight. In nonsmokers, the only

biochemical variable related to birth weight was cord vein red blood cell zinc.

Comment

Cigarette smoking during pregnancy results in a decrease in infant birth weight.[1] Smoking may actually be the most important risk factor for fetal growth disturbances. Thus it is not surprising that this phenomenon has recently been given the name "fetal tobacco syndrome."[8]

The mechanism for the decrease in birth weight is not completely understood but is thought to be due to hypoxia and vasoconstriction in the uterine vessels.[2,3] The results of this study suggest that cadmium and its interference with fetal zinc uptake may be related to the decrease in birth weight in infants of mothers who smoke during pregnancy.

While it is well known that zinc is needed for fetal growth in animals,[6] investigators have not always been able to show a relationship between maternal or fetal zinc status and birth weight in human infants. This was particularly true when the index of zinc status was serum zinc. For example, small for gestational age infants did not have serum zinc concentrations that were different from those of larger infants.[9-11] However, when leukocyte zinc was used as the index of zinc status, it could be shown that infants with prolonged intrauterine growth retardation had less zinc than normal infants.[11] We have shown in this study that cord vein red blood cell zinc is highly correlated with birth weight and have shown previously that it is negatively correlated with maternal smoking.[5] Thus it appears that if cord vein red blood cell zinc is used as the index of zinc status, the infant's zinc status may be related to both smoking and decreased birth weight.

Plasma zinc has been questioned as a valid indicator of zinc status since it represents only a small fraction of total body zinc.[6,11] Furthermore, it is known to decrease as the result of normal physiologic changes that occur during pregnancy.[6] Red blood cell zinc has been more difficult to measure but it probably represents a storage form of zinc and is a more stable index of zinc status.

The technique of multiple regression allows for control of confounding clinical variables and independent examination of the effects of related biochemical variables on an outcome variable. It was particularly useful in the present study because of the known confounding effects of clinical variables on birth weight and because several of the biochemical variables are related. For example both cord vein red blood cell zinc and whole blood cadmium are influenced by how much the mother smokes (that is, the thiocyanate level).[5] By use of multiple regression, it was possible to control clinical variables and for thiocyanate and then see if there was still a relationship between cadmium or zinc and birth weight. The results show that both maternal whole blood cadmium and cord vein red blood cell zinc predict birth weight independent of thiocyanate. The fact that these variables are altered by smoking suggests that this may be one explanation for the decreased birth weight in infants of smokers.

We have previously shown that increased maternal whole blood cadmium[12] and cadmium-zinc interaction due to smoking result in increased levels of both cadmium and zinc in the placenta and decreased cord vein red blood cell zinc in the infant.[5] The results of this study suggest that this biochemical interaction is clinically significant because increased maternal whole blood cadmium and decreased cord vein red blood cell zinc both are correlated with decreased birth weight.

Using simple correlations to look at smokers and nonsmokers separately suggested that the groups differ significantly in terms of factors related to birth weight. In nonsmokers, only cord vein red blood cell zinc was related to birth weight. In the smokers, negative relationships were found between birth weight and the cadmium and zinc variables. In other words, the more the mother smokes as measured by thiocyanate, the higher the levels of whole blood cadmium, placental cadmium, and placental zinc[5] and the lower the birth weight. This appears to be due to the trapping of zinc in the placenta, which results in less zinc in the infants' red blood cells and theoretically less zinc available for growth.

The ratio of placental zinc to placental cadmium was also positively related to infant birth weight in smokers. This is of interest because zinc is present in the placenta in much higher quantities than is cadmium. Zinc is measured in micrograms per gram of tissue, whereas cadmium is measured in nanograms per gram of tissue. Thus there is a thousandfold difference in the concentrations of the two metals. Nevertheless, the ratio of placental zinc to placental cadmium is related to birth weight. This attests to the importance of the cadmium-zinc interaction and to the toxicity of cadmium.

In addition to affecting birth weight directly, smoking may affect pregnancy in other ways that can also affect birth weight. For example, the length of gestation may be shortened. In order to minimize other effects on infant birth weight, standardized birth weight is often used to study the effect of smoking. This index is based on an expected mean birth weight for a historical population at a particular gestational age. By using stepwise multiple regression and controlling for the clinical factors such as gestational age, we accomplished the same thing. We were then able to look for a relationship between cord vein red blood cell zinc and the absolute size of the infant regardless of gestational

age. Our findings support the relationship between cadmium-zinc interaction and decreased birth weight in infants of smokers.

It has historically been difficult to prove causality between smoking and disease or smoking and lowered birth weight. However, with time, enough evidence has accumulated that little doubt remains as to the adverse effects of smoking. Similarly, it is difficult to show causality in this study between the cadmium and zinc variables and decreased birth weight. However, there are definitely relationships between the variables contributed primarily by smoking, that is, cadmium, and those known to be affected by smoking, that is, cord vein red blood cell zinc,[5] and birth weight. Furthermore, the relationships vary depending on the mother's smoking status during pregnancy; birth weights in smokers are predicted by the smoking variables. This was particularly evident in Table III. These associations make sense physiologically and are supported by the cadmium-zinc interaction recently reported in the human placenta by us[5] and previously reported in animal studies.[6] We hope that our studies will stimulate other trace element studies in pregnant smokers to confirm these relationships.

The results of this study suggest that zinc supplementation should be studied as a partial means of intervention for the pregnant smoker who cannot quit. The results of this study do not suggest that physicians should immediately start giving zinc to pregnant women who smoke. There are several reasons for this caveat: First, supplementation with zinc may disrupt the absorption of other essential elements, such as copper.[13] Second, the large amounts of iron or folic acid that are often given during pregnancy may interfere with the absorption of a zinc supplement.[14,15] Third, if the mothers are not zinc deficient, a supplement may have no effect on infant birth weight.[16] Thus a carefully controlled study that monitors zinc status and zinc, cadmium, and metallothionein levels in the placenta as well as birth weight needs to be done before universal zinc supplementation can be recommended for pregnant smokers.

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Pleural effusions and pulmonary hypoplasia

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Nine cases of fetuses with pleural effusions are presented in which the diagnosis was made by ultrasound before the thirtieth week of gestation. A ratio of lung span to hemithorax diameter was calculated and ranged from 0.44 to 0.77 (mean 0.60). At autopsy, pulmonary hypoplasia was confirmed in all cases by criteria based on the ratio of lung weight to total body weight. Because pleural effusions occurring in the midtrimester can be detected and may lead to pulmonary hypoplasia, consideration should be given to definitive in utero therapy when no other major fetal abnormality is detected. (AM J OBSTET GYNECOL 1987; 157:1252-5.)

Key words: Effusions, pulmonary hypoplasia, ultrasound

Increased use of real-time ultrasonography has resulted in early prenatal recognition of major fetal abnormalities. Prenatal detection of pleural effusions was first reported by Defoort and Thiery[1] in 1978; its association with pulmonary hypoplasia was later reported in 1981 by Bovicelli et al.[2] Subsequent reports have linked this condition with other anomalies or the syndrome of hydrops fetalis.[3, 4] The presence of pleural effusions is associated with a high incidence of fetal morbidity and mortality mainly because of the frequent coexistence of pulmonary hypoplasia. Although pulmonary hypoplasia is a histopathologic diagnosis, attempts have been made to correlate it with ultrasonographic findings.[6]

We now present a series of nine fetuses with significant pleural effusions diagnosed in utero before the thirtieth week of gestation. No prenatal management was undertaken and the effusions were quantified by use of the lung-thoracic ratio. Because all fetuses had pulmonary hypoplasia the natural history of this disorder and the relationship between the ultrasound findings of pleural effusions and the development of fetal lung hypoplasia are discussed.

Patients and methods

Table I is a summary of nine fetuses with bilateral pleural effusions studied in the Obstetrical Ultrasound Section of the Medical College of Georgia in the 2-year period from July 1984 to August 1986. During the initial ultrasound examination a detailed anatomic survey was performed and quantification of each pleural effusion was done. This measurement, described previously,[4] consists of a ratio between the lung span and the fetal hemithorax determined in transverse or longitudinal views. Also as part of the prenatal evaluation the following tests were performed: amniocentesis for fetal karyotype, delta optical density at 450 nm, and viral cultures. Analysis of maternal blood included a Kleihauer-Betke test, complete hemogram, blood type and antibody screen, glucose screen, and hemoglobin electrophoresis.

Results

Four pregnancies were electively terminated and one patient underwent induction of labor after intrauterine fetal death had been documented. In the other four cases, the fetuses were delivered alive after the spontaneous onset of labor. Autopsy was performed in each case and the results of each examination are presented in Table I. In three cases no anatomic abnormalities were found and there were no fluid accumulations in other serous cavities (Cases 1, 5, and 6). In Cases 2 and 8, no significant structural abnormalities were present, but a karyotypic disorder was found. In the remainder at least one other major anomaly was present although a direct association between these abnormalities and pulmonary hypoplasia could not be established.

All fetuses were found to have pulmonary hypoplasia as defined by the criteria of Reale and Esterly.[7] This definition consists of a ratio of total lung weight to total body weight that falls more than 2 SD below the normal value of 0.022 ± 0.002 and is constant throughout gestation.

Comment

The development of the fetal lung encompasses different stages that have characteristic anatomic findings:

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Table I. Summary of cases

Case No.	*Age (yr)*	*Gravidity*	*Parity*	*Gestational age at diagnosis (wk)*	*Gestational age at delivery (wk) and outcome*	*Lung-thoracic ratio (%)*	*Lung weight–body weight ratio**	*Prenatal findings*	*Postnatal findings*
1	22	2	1-0-0-1	26	32 SVD	46	0.003	Body edema Pleural effusions Ascites 46,XY	Severe hydrops Hypoplastic lungs
2	26	6	4-0-1-3	26	40 SVD	44.5	0.005	Body edema Pleural effusions Ascites 47,XY + 21	Severe hydrops Hypoplastic lungs
3	21	3	1-0-1-1	22	23 E.Ab.	62	0.009	Body edema Pleural effusions Ascites 46,XY	Severe hydrops Dysmorphic fascies Hypoplastic lungs Liver hyperplasia with focal areas of necrosis
4	18	1	—	24	24	64	0.002	Body edema Pleural effusions Neck cystic mass 46,XY	Severe hydrops Cystic hygroma Hypoplastic lungs
5	29	1	—	30	38 SVD	50 61.3 77.9	0.009	Pleural effusions 46,XY	Hypoplastic lungs
6	24	1	—	26	27.5 SVD	51.5	0.0012	Body edema Pleural effusions 46,XX	Hypoplastic lungs
7	25	3	2-0-0-2	25	28.5 IVD	60	0.0027	Fetal cell preparation 4% Body edema Pleural effusions Bilateral clubfoot Tachycardia 46,XY	Hypoplastic lungs Bilateral clubfoot Pena Shokeir type I
8	32	3	1-0-1-1	18	19 E.Ab.	63.6	0.0023	Body edema Pleural effusions Ascites 45,XO	Severe hydrops Hypoplastic lungs
9	23	2	1-0-0-1	18	19 E.Ab.	61.2	0.009	Body edema Neck cystic mass Pleural effusions 46,XY	Severe hydrops Hypoplastic lungs Cystic hygromas

SDV = Spontaneous vaginal delivery. E.Ab. = Elective abortion. IVD = Induced vaginal delivery.
*Expected = 0.022 ± 0.002.

(1) the glandular stage between the fifth and the seventeenth weeks, (2) the canalicular stage between the thirteenth and the twenty-fifth weeks, (3) the alveolar phase that occurs from the twenty-fourth week to approximately the eighth decade of life.[8] The intrauterine environment must provide a number of factors for these stages to proceed uneventfully; adequate amniotic fluid volume; presence of fetal breathing activity, intact muscular activity, and adequate intrathoracic and extrathoracic volumes that are capable of allowing the lung to expand. When one or more of these factors are not present or are significantly altered, the result is a spectrum of disorders ranging from minimal lung disease to severe pulmonary hypoplasia.[3, 4, 8]

In the present series, each fetus had bilateral pleural effusions that were quantified by the lung-thoracic ratio. In our institution any accumulation of fluid that can be quantified with this measurement is considered to be "significant." In Case 5 this measurement was repeated at 32 and 36 weeks' gestation with an objective decrease in the amount of the effusions (Fig. 1, *A* to *C*). Despite the reduction in pleural fluid in this fetus, the histopathologic findings were consistent with lung hypoplasia (Fig. 1, *D*). Therefore regardless of the cause the fluid accumulations appear to function as space-occupying lesions and restrict the development of the fetal lung, a sequence of events that was recognized by Potter in 1952 in fetuses with diaphragmatic hernias.[7]

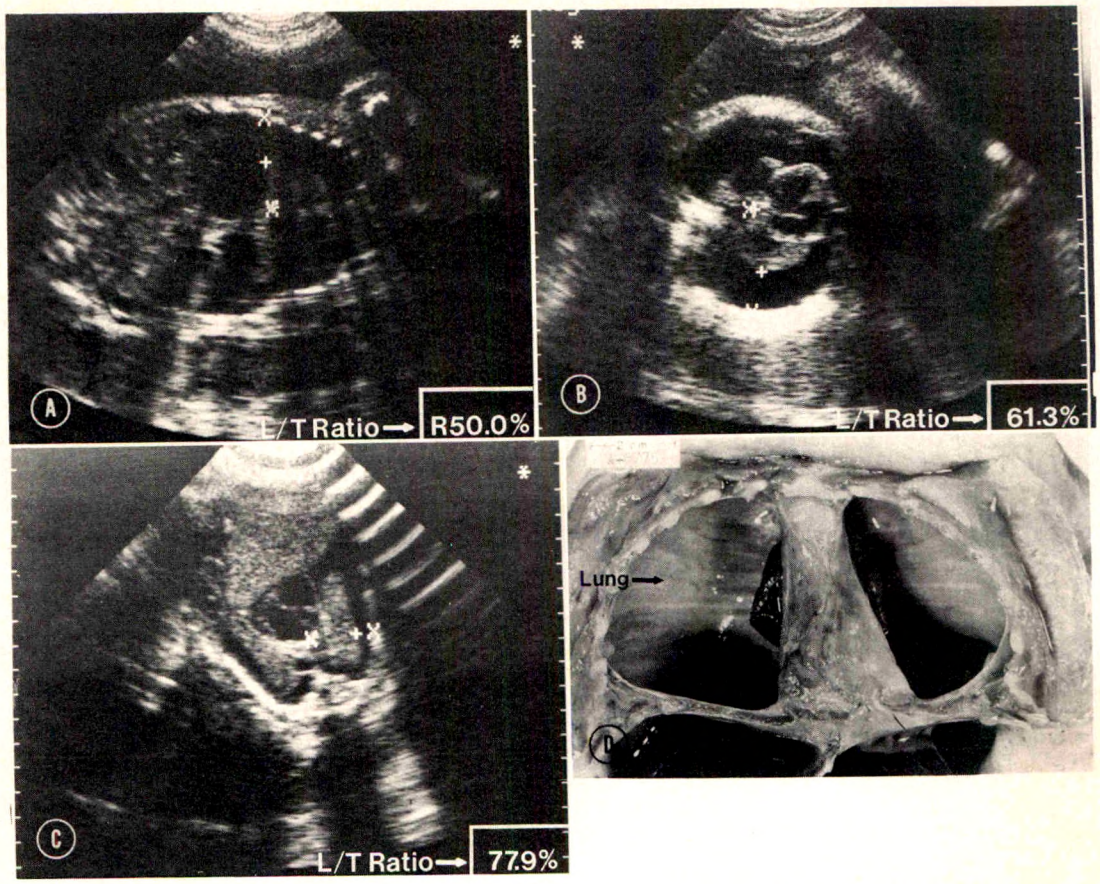


Fig. 1. Lung-thoracic ratios obtained at *(A)* 30 weeks', *(B)* 32 weeks', and *(C)* 36 weeks' gestation. Despite a reduction in the amount of pleural fluid the lungs were hypoplastic *(D)* with a ratio of lung weight to total body weight *(L/T ratio)* of 0.009.

However, it is clear that the sole presence of these effusions at birth is not enough to establish severe lung damage since adequate pulmonay function in some of these babies has been reported.[9] It seems that the gestational age at which these effusions develop may play a critical role in the development of severe and irreversible damage to the fetal lung.

Studies of morphologic lung development as well as a case report of persistent hypoplasia of the lung after repair of congenital diaphragmatic hernia suggest that such lesions appearing in the alveolar phase of lung development may not produce irreversible damage. Once the process is corrected, the development of alveoli may proceed although these may be significantly decreased in number.[8-10] These same studies and our histopathologic analysis of the nine hypoplastic lungs show that there are some changes in the bronchial tree and in the arterioles of hypoplastic lungs that indicate that the onset of this process occurred before the twenty-fourth to twenty-sixth week of gestation. These changes are reduced bronchial airway generation and increased muscularity of pulmonary blood vessels. Therefore it is suggested that if there are significant fluid accumulations in the pleural space before the alveolar phase and if this fluid accumulation persists throughout the remainder of pregnancy, the fetal lung could be irreversibly damaged.

Since 1982 at least five fetuses have been treated in utero by decompression of the thorax by means of either repeated pleural taps or placement of a pleural amniotic shunt.[5, 11-15] While the fetal outcomes are not always good, our experience suggests that untreated significant pleural effusions detected by the second trimester of pregnancy can lead to significant lung hypoplasia and perinatal death.

In summary, when pleural effusions are detected by ultrasound, a detailed anatomic survey of the fetus should be performed and accompanied by a complete antenatal workup as recommended for fetuses with nonimmune hydrops. If no major anomalies or significant karyotypic abnormalities are documented and the effusions are detected before the third trimester, prenatal therapy should be considered. Finally, aggressive neonatal support is mandatory at the time of delivery

since there is no absolute way of determining before birth which babies will have a pulmonary function incompatible with life.

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Antenatal diagnosis and management of monoamniotic twins

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Double survival of monoamniotic twins is rare. Three consecutive cases in which double survival occurred are presented. Accurate antenatal diagnosis, intensive fetal surveillance, and operative delivery should improve neonatal outcome. (Am J Obstet Gynecol 1987;157:1255-7.)

Key words: Monoamniotic twins, antenatal diagnosis

The incidence of monozygotic twins is approximately one in 250 pregnancies, with little racial or geographic differences worldwide, as opposed to dizygotic twins. Only 1% to 2% of monozygotic twins are monoamniotic. The perinatal mortality rate of monoamniotic twins has been reported to be as high as 50%.[1, 2] We present three consecutive cases of monoamniotic twins with double survival in all and discuss the antenatal diagnosis and management.

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Case reports

Case 1. A 22-year-old primigravida was transferred to the University of Connecticut Health Center at 33⅐ weeks' gestation because of preterm labor in a known twin gestation. Previous sonograms at 16, 20, and 24 weeks, as well as on adminssion, failed to reveal a dividing amniotic membrane. One placenta was seen, and amniotic fluid volume appeared normal around both female fetuses. No fetal anomalies were noted. At cesarean section both female fetuses occupied one amniotic sac. Twin B had a nuchal cord. The umbilical cords were intertwined and knotted and the placenta was monochorionic and monoamniotic (Fig. 1). Both

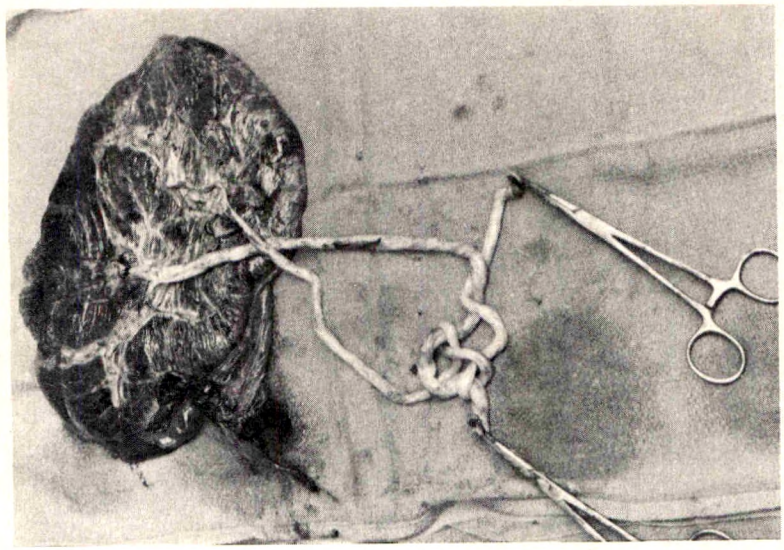


Fig. 1. Monochorionic, monoamniotic placenta with intertwining and knotting of cord.

fetuses and mother had uneventful postpartum courses and were discharged in good condition.

Case 2. A 28-year-old gravida 2, para 1 was discharged as having monoamniotic twins based on serial sonograms (one placenta, liked-sex female, absence of dividing membrane, and normal amniotic fluid around each fetus). Daily nonstress tests were begun at 27 weeks' gestation and were followed by biophysical profiles if nonreactive. At 34 weeks' gestation, a primary cesarean section was performed after confirmation of lung maturity by amniocentesis. Both twins were female, the cords were intertwined and knotted, and the placenta was monochorionic and monoamniotic. Mother and infants had unremarkable postpartum courses and were discharged on the fifth postpartum day.

Case 3. D. K. is a 36-year-old gravida 4, para 3 with known twin gestation. On ultrasound examinations performed at 14, 19, 20, and 24 weeks. a dividing membrane was never visualized while both female fetuses were surrounded by a normal amount of amniotic fluid. The patient was admitted to the University of Connecticut Health Center at 27 weeks' gestation for bed rest and daily fetal monitoring. Daily nonstress tests were performed on each fetus, and if nonreactive, were followed with biophysical profiles. Doppler umbilical artery velocimetry studies were performed every other day with an angioscan III. Ultrasound fetal measurements were obtained every 2 weeks to define the growth rate of each fetus and to look for signs of discordancy or twin-to-twin transfusion syndrome (ascites, pleural or pericardial effusions, and organomegaly). Betamethasone (12 mg intramuscularly) was administered weekly to enhance fetal pulmonary maturity.

Initial amniocentesis at 33 weeks revealed a lecithin/sphingomyelin ratio of 1.5 to 1. Repeat amniocentesis was performed at 34 weeks. Later that day occasional mild to moderate variable decelerations were noted on fetal heart rate monitoring. Before the result of the lecithin/sphingomyelin ratio was obtained, fetal bradycardia that was unresponsive to positional changes, intravenous fluids, and oxygen administration was noted in twin A. Immediate cesarean section was performed. At surgery only one amniotic sac was found. The umbilical cords were intertwined and knotted. Twin B had four loops of umbilical cord around the neck. Twin A, a 1720 gm female, had Apgar scores of 0 and 3 at 1 and 5 minutes, respectively, and required full cardiopulmonary resuscitation. Cord pH was 7.40 venous and 7.11 arterial. Twin B, a 1855 gm female, had Apgar scores of 1 and 7, at 1 and 5 minutes. Cord venous pH was 7.30 and arterial pH was 7.23.

Twin A was extubated on the first day of life and had an unremarkable neonatal course. Twin A had a hematocrit level of 45% at delivery and appeared to be the donor of a twin-to-twin transfusion syndrome. Twin B developed severe respiratory distress syndrome with classic radiographic findings and prolonged oxygen requirements. Twin B appeared to be the recipient of blood in the twin-to-twin transfusion syndrome with an initial hematocrit level of 61.7%. The lecithin/sphingomyelin ratio was subsequently reported as 2.7 to 1. Twin A was discharged from neonatal intensive care unit on the eleventh postnatal day and twin B on the fifty-sixth postnatal day. The mother had a benign postoperative.

Comment

If an original ultrasound examination fails to reveal a dividing amniotic membrane, a follow-up ultrasound examination should be performed at a tertiary care center. Before the diagnosis of monoamniotic twins can be entertained, the following criteria must be fulfilled:

(1) No dividing amniotic membrane should be visualized; (2) only one placenta should be seen; (3) both fetuses must have the same sex; (4) the fetuses must have adequate amniotic fluid surrounding them; and (5) both fetuses must move freely within the uterine cavity. This last point is important in avoiding the pitfall encountered in some diamniotic pregnancies in which the oligohydramnios in the one sac causes the membrane to be plastered against the fetus, thus making it difficult to visualize. This twin is usually flushed against the uterine wall, and this observation alone is indirect evidence of the presence of a dividing amniotic membrane. We recommend three consecutive ultrasound examinations to confirm the diagnosis.

Careful surveillance for the presence of fetal anomalies must be performed. Serial examinations every 2 weeks should be performed for assessment of fetal growth, discordancy, and twin-to-twin transfusion syndrome, which is a common problem in monoamniotic twins, and if significant may be a deciding factor for an earlier delivery. Cord entanglement should also be sought at each examination. We recommended hospitalization for bed rest and daily testing in all monoamniotic twins. Since the incidence of cord entanglement is great, we felt that changes in fetal heart tracings might precede intrauterine fetal demise as illustrated in case 2. Therefore daily nonstress tests are recommended.

With regard to the timing of the delivery, because of the rarity of the condition, no firm recommendations can be made based on data. However, since the perinatal mortality rate and incidence of cord entanglement are so high, we recommend intensive antenatal fetal surveillance from 25 until 32 weeks' gestation, after which time delivery should be undertaken. Amniocentesis may be helpful; however, even in the presence of a mature lung profile, one of the neonates may develop severe respiratory distress syndrome. If persistent variable decelerations or impaired fetal growth are noted, earlier delivery may be considered. We feel that cesarean section is the optimal route of delivery of monoamniotic twins. Unique complications of cord entanglement and the risk of fetal interlocking make vaginal delivery less than ideal.

In conclusion, monoamniotic twins represent a rare obstetric event with very high perinatal mortality. Accurate antenatal diagnosis, intensive antepartum fetal evaluation, and timed operative delivery in a tertiary care setting should improve the outcome of these infants.

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Blood pressure in the midtrimester and future eclampsia

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The purpose of this report was to investigate the potential usefulness of average mean arterial pressure, maximal mean arterial pressure, and maximal diastolic pressure during the second trimester in predicting the development of eclampsia in 207 nulliparas and 20 multiparas with eclampsia. In the nulliparas, both the mean arterial pressure and the maximal mean arterial pressure during the second trimester were ≥90 mm Hg in 22% and 34% of the patients, respectively. For the multiparas, the percentages with ≥90 mm Hg were 30% and 35%, respectively. Only 8.7% of nulliparas with eclampsia had a maximal diastolic pressure during the second trimester ≥80 mm Hg and no patient had a diastolic pressure of ≥90 mm Hg. A review of the literature suggests that the mean arterial pressure observed during the second trimester has poor predictive value for future development of preeclampsia-eclampsia. We conclude that there is no correlation between second-trimester blood pressure recordings and subsequent eclampsia. (Am J Obstet Gynecol 1987;157:1258-61.)

Key words: Mean arterial pressure, eclampsia

Routine recordings of blood pressure, as ascertained by a variety of medical personnel, are usually the only data available for the evaluation of antepartum blood pressures in a busy obstetric service. No analysis of such data could be regarded as a scientific study of blood pressure, and yet they have some value. It has been alleged, for instance, that they are useful in the prediction of later preeclampsia.

Fallis and Langford[1] suggested that the level of blood pressure before the onset of toxemia might be predictive of the development of hypertension in late pregnancy. They studied 113 primigravidas, 95 of whom were black, and observed that the average mean arterial pressure had been 92.8 mm Hg in those patients who later developed toxemia and 83.0 mm Hg in those who did not (weighted averages derived from two sets of data in the paper).

Page and Christianson[2] averaged all the mean arterial pressures calculated from observations made during the second trimester for each of 14,833 women enrolled in the Kaiser-Permanente Foundation Health Plan in the San Francisco Bay Area and found that the later incidences of proteinuria, preeclampsia, and hypertension increased progressively with higher average mean arterial pressures during the second trimester, with sharp increments when the average mean arterial pressure was 95 mm Hg or higher. Among 686 women with an average mean arterial pressure during the second trimester of 95 mm Hg or higher, 5.4% developed proteinuria of at least two plus, 14.2% developed preeclampsia, and 25.9% had increases in mean arterial pressure to at least 110 mm Hg in the third trimester. The extent of overlapping of the three groups is unclear; presumably the women with proteinuria were included among the patients with preeclampsia, who may have been included among the women with hypertension.

Several such studies have purported to show that preeclampsia can often be predicted from average mean arterial pressure of the second trimester of 90 mm Hg or higher but with high percentages of false-positive and false-negative tests.[3-7] A basic weakness in such studies is that most of the diagnoses have been mild preeclampsia, a diagnosis that is erroneous in most multiparas and in a large proportion of primigravidas, many of whom have transient hypertension rather than preeclampsia.

The acid test of the efficacy of prediction is how well the mean arterial pressure of the second trimester foretells eclampsia. This article assesses the predictive value of blood pressure in the second trimester for the development of eclampsia in two large series, one during the period 1931 through 1951 and the other from 1977 through 1986. Relevant information is available for a total of 227 cases of eclampsia.

Material and methods

Cases of eclampsia that occurred in the Margaret Hague Maternity Hospital (1931 to 1952) in Jersey City, New Jersey, and the E. H. Crump Women's Hospital (1977 to 1986) in Memphis, Tennessee, were used for the retrospective analysis. Among the 303 cases at Mar-

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Table I. Average mean arterial pressure (mm Hg) during the second trimester in two groups of patients with eclampsia

Group	*No. of cases*	*Mean ± 1 SD*	*% with*	
			90-94 mm Hg	*≥95 mm Hg*
Nulliparas				
Margaret Hague	85	85.3 ± 7.3	20	7
E. H. Crump	122	81.2 ± 8.1	15	3
Total	207	82.9 ± 7.7	17	5
Multiparas				
Margaret Hague	9	82.8 ± 7.3	33	0
E. H. Crump	11	82.9 ± 9.6	18	9
Total	20	82.9 ± 9.0	25	5

Table II. Maximal mean arterial pressure in the second trimester in two groups of patients with eclampsia

Group	*No. of cases*	*Mean ± 1 SD*	*% with*	
			90-94 mm Hg	*≥95 mm Hg*
Nulliparas				
Margaret Hague	85	86.1 ± 6.30	28	14
E. H. Crump	122	85.9 ± 6.1	20	9
Total	207	86.0 ± 6.20	23	11
Multiparas				
Margaret Hague	9	86.2 ± 4.1	33	0
E. H. Crump	11	86.9 ± 8.9	27	9
Total	20	86.6 ± 8.0	30	5

garet Hague, recordings of blood pressures in the second trimester were available for only 85 nulliparas and nine multiparas. Among the 210 cases at E. H. Crump, recordings of blood pressures in the second trimester were available for 122 nulliparas and 11 multiparas. All patients with known chronic hypertension or renal disease were excluded from the analysis.

Mean arterial pressures were calculated by adding one third of the pulse pressure to the diastolic reading. For every patient, both average mean arterial pressure during the second trimester and maximal mean arterial pressure during the second trimester, as well as the maximal diastolic blood pressure in the second trimester were evaluated as predictors of future eclampsia. Results of various blood pressures studied were analyzed for each hospital and the two hospitals combined.

Results

Table I summarizes the average mean arterial pressure during the second trimester and the percentage of patients with levels ≥90 mm Hg in the two hospitals and the total number of patients with eclampsia for both nulliparas and multiparas. It is interesting to note that the findings in both groups are similar even though the patients were from two different regions at two different time periods. Overall, only 22% of nulliparas and 30% of multiparas had an average mean arterial pressure during the second trimester ≥90 mm Hg before the development of eclampsia. There were no immediate deaths from eclampsia at E. H. Crump, while five such deaths occurred at Margaret Hague. The average levels of mean arterial pressure during the second trimester in these deaths were 74, 83, 91, and 96 mm Hg in the nulliparas and 92 in the one multipara. Some of the multiparas in the Margaret Hague group may have had underlying and unrecognized chronic hypertension, since at follow-up seven of the eight surviving pregnant patients had chronic hypertension.

Table II summarizes the maximal mean arterial pressure in the second trimester and percentages of patients with levels ≥90 mm Hg in the two groups of patients. The findings in both groups are again almost identical. Overall, only 34% of nulliparas and 35% of multiparas had a maximum mean arterial pressure during the second trimester ≥90 mm Hg, an incidence that is slightly lower than the 39% found in whole hospital populations by Friedman and Neff.

Table III shows the maximal diastolic pressure during the second trimester in both groups of patients with eclampsia. There were no differences between patients from the different hospitals and between nulliparas and multiparas. It is apparent that the maximal diastolic pressure during the second trimester has the worst predictive value regarding future development of eclamp-

Table III. Maximal diastolic pressure in the second trimester in two groups of patients with eclampsia

Group	No. of cases	Mean ± 1 SD	% with		
			80-84	85-89	90-94 mm Hg
Nulliparas					
Margaret Hague	85	71.7 ± 9.8	6	3	0
E. H. Crump	122	71.4 ± 8.0	7	2	0
Total	207	71.5 ± 9.0	6.3	2.4	0
Multiparas					
Margaret Hague	9	71.6 ± 9.0	22	0	11
E. H. Crump	11	72.9 ± 9.1	18	9	0
Total	20	72.2 ± 9.1	20	5	5

sia. Overall, only 8.2% of nulliparas had a maximal diastolic pressure during the second trimester ≥80 mm Hg, and no patient in this group had a level >90 mm Hg. In the multiparas, 30% of patients had a maximal diastolic pressure ≥80 mm Hg, but only 5% had a level of ≥90 mm Hg. This finding may reflect the fact that some of the multiparas may have had underlying and unrecognized chronic hypertension.

Comments

The findings of this study reveal that only 22% of 207 nulliparas with eclampsia have an average mean arterial pressure during the second trimester ≥90 mm Hg, whereas 30% of 20 multiparas with eclampsia have had such a finding. It seems that an average mean arterial pressure during the second trimester ≥90 mm Hg is not sensitive enough to predict the future development of eclampsia. The same holds true for a maximal mean arterial pressure value of ≥90 mm Hg, since only 34% of nulliparas with eclampsia and 35% of multiparas with eclampsia had such a value. As for the maximal diastolic pressure recording in the second trimester, this test appears to be the poorest for the future development of eclampsia in the nulliparas. In fact, only 8.7% of nulliparas with eclampsia had a value of ≥80 mm Hg, and no patient had a diastolic pressure of ≥90 mm Hg in the second trimester. On the other hand, 30% of multiparas with eclampsia had a maximal diastolic pressure during the second trimester ≥80 mm Hg. This finding may reflect the fact that some of these patients might have had underlying and unrecognized chronic hypertension.

Moller and Lindmark[8] reported on 74 patients who developed eclampsia in Sweden during the years 1967 to 1980. Only five patients did not have regular prenatal care visits, and 88% had their first prenatal visit before 16 weeks' gestation. The average blood pressure in these patients at initial visit was 120/70 mm Hg. Thirty-three patients had eclampsia before 37 weeks' gestation and 41 had eclampsia at ≥37 weeks. The authors found that only 39% of patients who had eclampsia before 37 weeks and 16% of patients who had eclampsia beyond 37 weeks' gestation had a diagnosed elevation of blood pressure (≥140/90 mm Hg) 4 days before the first eclamptic convulsion. It can be concluded from these findings that most patients wtih eclampsia had a maximal diastolic pressure of <90 mm Hg before the onset of eclampsia. In addition, several of these women had an average mean arterial pressure during the second trimester of <90 mm Hg.

The onset of eclampsia may be abrupt without any warning signs or symptoms in about 20% of cases. In studying the possible factors leading to the development of eclampsia in 66 patients, Campbell and Templeton[9] found that the onset was abrupt in 11 patients (16.7%). These patients had normal blood pressure and absent proteinuria within 1 week before the onset of convulsions. In addition, Dieckmann[10] found that 22% of patients with eclampsia had a systolic blood pressure <140 mm Hg before the onset of convulsions. Recently Clark et al.[11] compared the hemodynamic findings in three patients with eclampsia and four patients with severe preeclampsia. They found similar hemodynamic findings in the two groups except for a significantly lower plasma colloid osmotic pressures in the patients with eclampsia. This suggests that eclampsia is a multifactorial disease and that current techniques to predict subsequent eclampsia are indeed lacking.

In summary, the level of the mean arterial blood pressure during the second trimester is a poor predictor for the future development of eclampsia and might give the clinician a false sense of security when it is negative (<90 mm Hg). We believe that if an increase in the levels of mean arterial pressure during the second trimester predict anything, it is transient hypertension rather than eclampsia.

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Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons

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Effects of maternal hypoxia on fetoplacental vascular resistance in the human placenta were investigated in an in vitro model in which single anatomic subunits (cotyledons) from term placentas were perfused at constant flow through both fetal and maternal circuits by means of a physiologic salt solution containing dextran. Acute reduction of oxygen tension in the maternal perfusate induced prompt fetoplacental vasoconstriction that recovered rapidly on restoration of oxygen to the perfusate. The response, hypoxic fetoplacental vasoconstriction, could be repeatedly demonstrated in the same cotyledon. The time course of hypoxic fetoplacental vasoconstriction was inversely related to oxygen tension of maternal arterial and maternal and fetal venous perfusates. Maternal and fetal venous perfusate pH and P_{CO_2} did not change during the response. It is concluded that hypoxic fetoplacental vasoconstriction is triggered by decreased oxygen availability. It is suggested that hypoxic fetoplacental vasoconstriction may play a role in local regulation of human fetoplacental blood flow in vivo and may contribute to poor fetal prognosis in preeclampsia. (AM J OBSTET GYNECOL 1987;157:1261-6.)

Key words: Hypoxia, fetoplacental vasoconstriction, placental perfusion

A major function of the placenta is the transfer of oxygen and nutrients from maternal to fetal blood. Regulation of fetal blood flow through the placenta is not understood, but since fetoplacental vessels are not innervated, local and/or humoral mechanisms are likely to be important. In the near-term sheep, occlusion of maternal placental blood flow reduced adjacent fetal placental flow,[1] and similarly ligation of a portion of the umbilical vasculature resulted in a decrease in adjacent maternal placental flow,[2] which support the concept of local regulatory interaction between fetal and maternal placental circulations in this species.[1] The ovine placenta is epitheliochorial and differs from the hemochorial human placenta in anatomic separation of maternal and fetal blood. Whether fetoplacental blood flow in human pregnancy is influenced by uteroplacental blood flow is not known. Demonstration of such an interaction in the human placenta would have potential significance in understanding how fetoplacental vascular resistance is controlled in normal pregnancy and in preeclampsia, a common disorder of pregnancy.

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Preeclampsia is typically associated with placental ischemia,[3] and chronic hypoxia has been suggested as a factor contributing to the narrowing and obliteration of fetal arteries observed in placentas from patients with preeclampsia.[4] In the present study we asked whether uteroplacental hypoxia might affect human fetoplacental vascular resistance and thus modify fetal blood flow through the placenta.

Effects of human placental hypoxia could not be determined in vivo because studies of placental function in vivo are limited to therapeutic and noninvasive procedures. Moreover, we did not have access to an animal model of the villous hemochorial placenta, such as the subhuman primate. We therefore used an in vitro model, the isolated dual-perfused cotyledon from human term placenta.[5] The human placenta is made up of a number of discrete anatomic subunits or cotyledons that can be isolated and perfused via both fetal and maternal circuits.[5] Maternal circulation in the human placenta is not confined to a separate vasculature but flows into the intervillous space and directly bathes the villi of the fetal placenta. In the placental model perfused in vitro, cannulas inserted through the maternal surface of the cotyledon deliver perfusate to the intervillous space[5]: Careful placement of the cannulas is necessary to achieve adequate perfusion of the intervillous space. Using this preparation, we have determined dose-dependent effects of autacoids and humoral agents on fetoplacental vascular resistance.[6-8] In this article we report that perfusion of the maternal circuit of the in vitro placental cotyledon with hypoxic medium causes reversible fetoplacental vasoconstriction.

Material and methods

Placentas from normal full-term pregnancies were obtained immediately after delivery. A single cotyledon from each placenta was set up for constant flow perfusion of the fetal circuit and intervillous space with Earle's salt solution to which 40 gm/L of dextran (approximate molecular weight 40,000) was added.[5-7] The upper chamber of the perfusion apparatus was open to allow access to the maternal surface of the perfused cotyledon. Preparation and perfusates were maintained at 37° C. Perfusate flow rates were monitored with Gilson No. 1 flowmeters. Fetal perfusate was gassed with 94% nitrogen, 6% carbon dioxide. Normoxic maternal perfusate was gassed with 95% oxygen, 5% carbon dioxide, and hypoxic maternal perfusate was gassed with 95% nitrogen, 5% carbon dioxide in a separate reservoir. Effective placement of maternal cannulas was monitored by measurement of fetal venous gas tensions and pH. Maternal flow rate was increased, if necessary, to achieve fetal venous pH of 7.30 to 7.35, P_{CO_2} of 39 to 47 mm Hg, and P_{O_2} >100 mm Hg. Gas and pH values of perfusates were measured with a Radiometer BMS 3 blood gas analyzer. Maternal and fetal arterial and venous perfusates were sampled with glass syringes from ports in the perfusion lines. Maternal venous perfusate was sampled by a syringe from the perfusate outflow above the maternal surface adjacent to the maternal cannulas. Perfusion pressures were monitored via Statham P23ID transducers and recorded on a Gilson ICT-2H duograph; perfusion pressure of the fetal circuit was used as an index of fetal vascular resistance.

Experimental procedure. Control conditions of normoxic maternal perfusion were established. Hypoxia was then imposed by substitution of the normoxic maternal perfusate by hypoxic maternal perfusate for 8 to 15 minutes; perfusion with normoxic maternal perfusate was then reinstituted. Transit time from reservoir to intervillous space was 1 to 2 minutes. A period of 3 to 4 minutes was allowed to elapse between recovery and the next hypoxic challenge of the same duration. At the end of each experiment, a 1% solution of Coomassie blue in saline solution was infused via the fetal arterial cannula, and the cotyledon demarcated by the dye was dissected and weighed. Perfusate flow rates are expressed as ml min^{-1} gm^{-1}.

Perfusion medium. Earle's salt solution had the following composition (mmol/L): NaCl 116.4; $NaHCO_3$ 26.2; KCl 5.36; $CaCl_2$ 1.80; $MgSO_4$ 0.81; NaH_2PO_4 1.01; and glucose 5.5. NaCl was Fisher biologic grade. Other salts were the best analytic grade available. Dextran, molecular weight 38,000 to 42,000, was obtained from Chemical Dynamics Corp., New Jersey, or ICN, Ohio.

Data analysis. Results are given as mean ± standard error of the mean. Differences between means of perfusate gas and pH values before and during maternal hypoxia were evaluated by a one-way analysis of variance F test with 4 and 15 degrees of freedom; BMDP7D[9] statistical software was used for computations. Correlation coefficients, partial correlation coefficients,[10] and multiple-regression analysis were computed with BMDP6R and BMDP2R statistical software.[9]

Results

Weights of the 44 cotyledons used in the study ranged from 8 to 27 gm with a mean of 14.8 ± 0.7 gm. Control fetal vascular resistance in the preparations was 115 ± 6 mm Hg min gm ml^{-1}, and mean flow rates of maternal and fetal perfusates were 1.35 ± 0.005 and 0.41 ± 0.02 ml min^{-1} gm^{-1}, respectively. Perfusates had the following control gas and pH values: maternal arterial: pH 7.393 ± 0.003, P_{CO_2} 34.8 ± 0.3 mm Hg, P_{O_2} 540 ± 4 mm Hg (n = 16 to 18 cotyledons); maternal venous: pH 7.358 ± 0.007, P_{CO_2} 40.6 ± 0.4 mm Hg, P_{O_2} 355 ± 14 mm Hg (n = 11 to 14 cotyledons); fetal arterial: pH 7.342 ± 0.007, P_{CO_2} 42.9 ± 0.6 mm Hg, P_{O_2} 27 ± 2 mm Hg (n = six cotyledons); and fetal venous: pH 7.357 ± 0.003, P_{CO_2} 42.6 ± 0.4

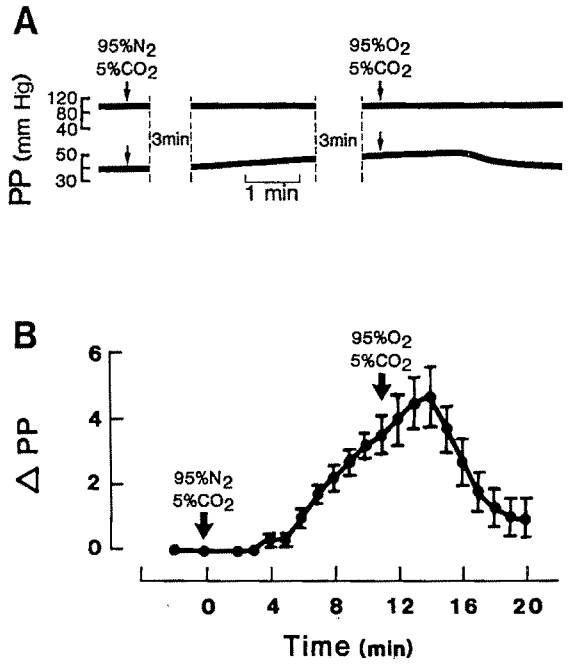


Fig. 1. A, Typical effects on maternal *(upper tracing)* and fetal *(lower tracing)* perfusion pressure observed when the maternal circuit of the human placental cotyledon was perfused with hypoxic medium. *Arrows* indicate the start of 9 minutes of hypoxic maternal perfusion (95% N_2, 5% CO_2) and restoration of normoxic perfusion (95% O_2, 5% CO_2). **B,** Time course of averaged pressor responses to three sequential hypoxic stimuli of 10-minute duration in one cotyledon. (Δ *PP* = Change in perfusion pressure [mm Hg], *bars* indicate standard error of the mean.)

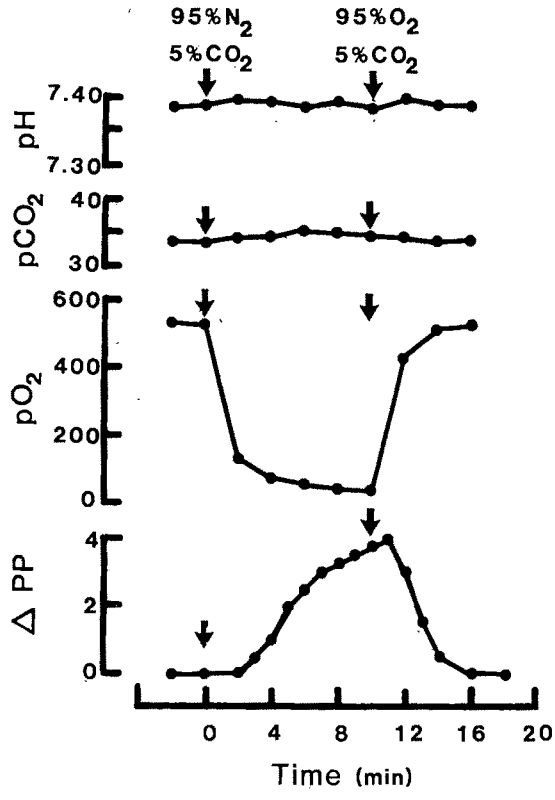


Fig. 2. Time courses of a typical fetal pressor response and associated maternal arterial perfusate gas and pH values induced by 10-minute hypoxic challenge in a single cotyledon. *Arrows* indicate start of hypoxic maternal perfusion (95% N_2, 5% CO_2) and normoxic maternal perfusion (95% O_2, 5% CO_2). (Δ *PP* = Change in fetal perfusion pressure [mm Hg].)

mm Hg, Po_2 198 ± 5 mm Hg (n = 42 cotyledons). The mean arteriovenous increase in fetal perfusate Po_2 of approximately 170 mm Hg indicated that a significant fraction of oxygen entering the maternal side of the cotyledon was transferred across the placenta.

Perfusion of the maternal circuit of the cotyledons with hypoxic perfusate induced prompt reversible vasoconstriction of the fetoplacental vasculature as evidenced by a rise in fetal perfusion pressure 2 to 4 minutes after perfusion with the hypoxic perfusate commenced, which was followed by a drop in pressure 2 to 4 minutes after delivery of normoxic maternal perfusate was reinstituted (Fig. 1, *A*). Fetoplacental vasoconstriction induced by maternal hypoxia could be repeatedly elicited in the same cotyledon (Fig. 1, *B*). Return to baseline perfusion pressure was complete or nearly complete with recovery from each response (Figs. 1, *B*, 2, and 3). This reversible response is described as hypoxic fetoplacental vasoconstriction.

Development of the fetal pressor response to maternal hypoxia and recovery of baseline perfusion pressure paralleled changes in the oxygen tension of maternal and fetal perfusates. Fig. 2 shows the fall in maternal arterial Po_2 from 540 to 25 mm Hg that accompanies the onset of hypoxic fetoplacental vasoconstriction and the subsequent return to control level Po_2 associated with recovery from the response. The onset of hypoxic fetoplacental vasoconstriction and recovery from hypoxic fetoplacental vasoconstriction were also reflected in the fall and rise, respectively, of Po_2 in both maternal and fetal venous perfusates as shown for a typical experiment in Fig. 3. Oxygen tensions in both maternal and fetal venous perfusates varied significantly during hypoxic fetoplacental vasoconstriction (fetal venous $F = 490.48$, $p < 0.0001$; maternal venous $F = 70.85$, $p < 0.0001$). As indicated in Fig. 3, the pressor response commenced as the fetal venous Po_2 fell below approximately 100 mm Hg and continued to rise as fetal venous Po_2 fell to below 40 to 50 mm Hg 2 to 4 minutes after reinstitution of normoxic perfusion. No significant change from prehypoxic maternal venous or fetal venous pH was observed during hypoxic fetoplacental vasoconstriction (compare Fig. 3; fetal venous, $F = 0.12$, $p = 0.97$; maternal venous, $F = 0.47$, $p = 0.76$). No significant change from prehypoxic values occurred in either fetal venous

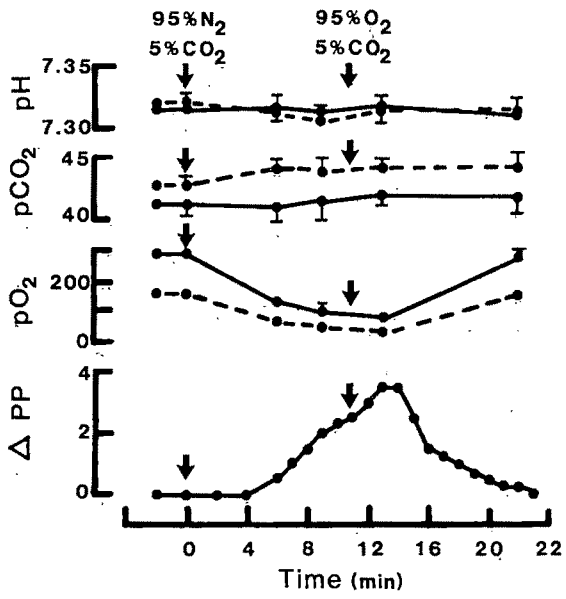


Fig. 3. Time courses of fetal *(broken lines)* and maternal *(solid lines)* venous perfusate gas and pH values induced by three hypoxic stimuli of 11-minute duration and a typical pressor response in the same cotyledon. *Arrows* indicate start of maternal hypoxic (95% N_2, 5% CO_2) and maternal normoxic (95% O_2, 5% CO_2) perfusion. Gas and pH values are mean ± standard error of the mean; where not shown standard error of the mean is within the data point. (Δ *PP* = Change in perfusion pressure [mm Hg].)

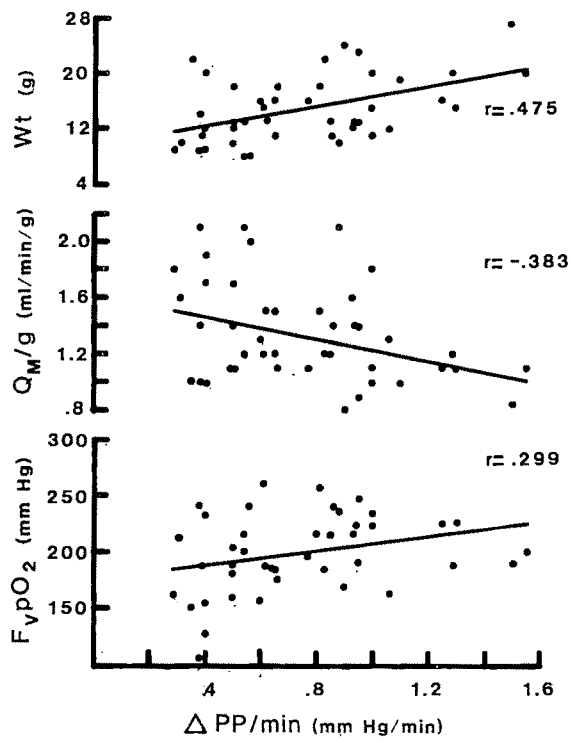


Fig. 4. Relationships between the normalized fetal pressor response to hypoxic challenge (Δ PP min^{-1}, mm Hg min^{-1}) and each of three different parameters of perfusion: cotyledon weight *(wt)*, maternal flow per gm (Q_M gm^{-1}, ml min^{-1} gm^{-1}), and control (i.e., before hypoxic challenge) fetal venous P_{O_2} ($F_vP_{O_2}$) in 41 different cotyledons. (*r* = simple correlation coefficient.)

P_{CO_2} or maternal venous P_{CO_2} during the response (fetal venous F 0.25, p = 0.90; maternal venous F = 0.28, p = 0.89) (Fig. 3). Furthermore, in two preparations subjected to repeated hypoxia, neither P_{CO_2} nor pH of fetal venous perfusate measured before hypoxic vasoconstriction differed significantly from pH and P_{CO_2} measured during peak hypoxic vasoconstriction within each preparation (Table I). However, fetal venous P_{O_2} during hypoxic constriction fell to less than 25% of the prehypoxia control level (Table I). Similarly, comparison of gas tensions and pH values of maternal venous perfusates before and during repeated hypoxic fetoplacental vasoconstriction in three different cotyledons showed no difference in pH and P_{CO_2} before and during the responses but showed a twofold to threefold fall in P_{O_2} (data not shown).

In 55% of the cotyledons studied, the maximum extent of vasoconstriction to hypoxic stimuli of 9- to 11-minute duration was observed with the first hypoxic stimulus, but in 45% of the cotyledons the magnitude of the response increased with repeated hypoxia until the maximum response observed in the cotyledon was achieved with the third or fourth stimulus. The reason for this difference between cotyledons is not presently understood. The maximum hypoxic vasoconstriction for each cotyledon, which was normalized as pressure increase per minute of hypoxic stimulus, ranged from 0.3 to 1.6 mm Hg min^{-1}, with a mean of 0.76 ± 0.05 mm Hg min^{-1} (n = 44 cotyledons). It was thought that the variation between cotyledons in the normalized response to maternal hypoxia might be related to parameters of the preparation such as cotyledon weight, maternal and fetal flow rates (ml min^{-1} gm^{-1}), control fetoplacental vascular resistance (mm Hg min gm ml^{-1}), or control perfusate gas tensions and pH. Regression analysis showed that the normalized hypoxic pressor response indeed correlated with cotyledon weight, maternal flow rate, and control (normoxic) fetal venous P_{O_2}, as shown in Fig. 4 and by the simple correlation coefficients given in the figure. However, the response did not correlate with fetal venous pH (r = 0.047), fetal venous P_{CO_2} (r = 0.09), fetal perfusate flow rate (r = 0.141), or fetoplacental vascular resistance (r = 0.036). Further statistical analysis was performed to elucidate how the normalized hypoxic pressor response was related to cotyledon weight, maternal flow rate, and normoxic fetal venous P_{O_2}. Specifically, partial correlation coefficients were computed to assess the extent to which each of these three parameters were (uniquely)

Table I. Comparison of fetal venous perfusate pH, P_{CO_2}, and P_{O_2} during normoxic maternal perfusion before hypoxic challenge with pH, P_{CO_2}, and P_{O_2} at the peak pressor response to hypoxic maternal perfusion in two dual-perfused placental cotyledons

Cotyledon No.	*Duration of hypoxia (min)*	*n**	*Fetal venous pH*		*Fetal venous P_{CO_2}*		*Fetal venous P_{O_2}*	
			Control	*Hypoxia*	*Control*	*Hypoxia*	*Control*	*Hypoxia*
1	10	7	7.372 ± 0.001	7.373 ± 0.001	41.3 ± 0.3	41.3 ± 0.2	196 ± 2	47 ± 3
2	11	4	7.320 ± 0.005	7.316 ± 0.01	43.1 ± 0.7	44.2 ± 1.0	155 ± 2	31 ± 2

Values are mean ± SEM.

*Number of sequential hypoxic stimuli; replicate three to five measurements of pH, P_{CO_2}, and P_{O_2} were made in control and hypoxia perfusates sampled before and during each pressor response.

related to the pressor response while statistically controlling the influences of the other two. Partial correlation coefficients of the pressor response with fetal venous P_{O_2}, cotyledon weight, and maternal flow rate (ml min^{-1} gm^{-1}) were 0.449, 0.325, and −0.087, respectively, thus suggesting that control fetal venous P_{O_2} was the parameter most highly related to the normalized pressor response independent of the other two parameters. However, the partial correlation coefficient of 0.325 associated with cotyledon weight suggests that it too may be uniquely related to the magnitude of the normalized hypoxic pressor response. These conclusions were confirmed by multiple-regression analysis for the normalized pressor response ($R = 0.6035$). Of the three individual regression coefficients, those for fetal venous P_{O_2} ($p < 0.01$) and cotyledon weight ($p < 0.05$) were statistically significant.

Comment

In this study the effect of acute uteroplacental hypoxia on human fetoplacental vascular resistance has been investigated with the isolated dual-perfused placental cotyledon as an in vitro experimental model. Several parameters of this in vitro model resembled those reported for the in vivo placenta. Specifically, maternal and fetal flow rates of 1.35 ± 0.005 and 0.41 ± 0.02 ml min^{-1} gm^{-1} were similar to flow rates calculated for maternal and umbilical blood in vivo, that is, 1.0 and 0.6 ml min^{-1} gm^{-1}, respectively (calculated using reported uteroplacental[11] and umbilical[12] flow rates and assuming a placental weight of 500 gm), and gas tensions and pH of the fetal arterial perfusate approximated those found in vivo.[13] With this model we demonstrated that maternal hypoxia induced reversible fetoplacental vasoconstriction. The response was inversely related to maternal arterial perfusate oxygen tension and was associated with a reversible drop in P_{O_2} of maternal and fetal venous perfusates. However, neither pH nor P_{CO_2} of the venous perfusates changed during the hypoxic response, which suggests that the mechanism of the response does not involve change in glucose metabolism. We conclude that decreased oxygen availability is the primary triggering factor in hypoxic fetoplacental vasoconstriction since oxygen tension was the only variable changed.

A fivefold variation in the size of the normalized hypoxic vasoconstriction between different cotyledons was observed and was shown by statistical analysis to be correlated with two prestimulus perfusion parameters, control fetal venous P_{O_2} and cotyledon weight. These findings suggest that cotyledons that transferred oxygen most effectively across the placenta and larger cotyledons were more sensitive to maternal hypoxia. Reasons for the increased sensitivity are unknown. However, it may be speculated that general function was better preserved in those cotyledons that transferred oxygen to the fetal circulation more effectively, and that as a result sensitivity to lack of oxygen was greater. On the other hand, the basis of the positive correlation between cotyledon size and sensitivity to maternal hypoxia is less readily explained. It is unlikely to be the result of better maternal perfusion in the larger cotyledons, since analysis of interrelationships of perfusion parameters under control conditions has shown that maternal flow rate (ml min^{-1} gm^{-1}) correlated negatively with cotyledon weight.[14] Again, it may be that function is better preserved at term in the larger cotyledons.

Although we have demonstrated hypoxic fetoplacental vasoconstriction in an artificially perfused placental preparation in vitro, the response could be important in vivo, for example, in acute local blood flow regulation in which it may result in a redistribution of fetoplacental blood flow from hypoxic to normoxic or better perfused regions of the placenta. In this regard, the response may be analogous to the phenomenon of hypoxic pulmonary vasoconstriction, which is important in the adjustment of ventilation-perfusion ratios in the lung. We have shown that verapamil and lipoxygenase inhibitors antagonize hypoxic fetoplacental vasoconstriction,[15] which suggests that the response is mediated, at least in part, by release of lipoxygenase products.

Fetoplacental vasoconstriction in response to maternal hypoxia has not been previously described, although it is reminiscent of the reduction of fetal placental blood flow observed 24 hours after maternal placental occlusion in the near-term sheep.[1] However, we know of no evidence that the ovine response to occlusion of maternal placental flow occurs acutely and reversibly. An acute pressor response to abolition of maternal perfusion can be demonstrated in the in vitro placental preparation and is reversible on restoration of maternal perfusion.[16]

Hypoxic fetoplacental vasoconstriction in the in vitro cotyledon resulted from acute change in maternal oxygen tension. It seems possible that in vivo, chronic placental hypoxia such as has been postulated to occur in preeclampsia[3, 4] may cause a chronic vasoconstrictor response that may contribute to fetal mortality and growth retardation associated with this disease. Furthermore, release of hypoxia-induced vasoconstrictive mediators may be important in the etiology of preeclampsia.

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Haptoglobin helps diagnose the HELLP syndrome

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In a case of the HELLP syndrome, anemia was absent, and bilirubin and lactic dehydrogenase levels were normal. In combination with minimal microangiopathic changes in red cell morphology, a decrease in serum haptoglobin was useful to corroborate the presence of intravascular hemolysis. (AM J OBSTET GYNECOL 1987;157:1267.)

Key words: HELLP syndrome, serum haptoglobin level

The HELLP syndrome (hemolysis, elevated liver function tests, and low platelets) has become a recognized cause of antenatal thrombocytopenia.[1] Diagnostic uncertainty may arise if one component of the classic triad seems to be absent. This report illustrates a case of the HELLP syndrome in which a decrease in serum haptoglobin level confirmed the presence of hemolysis despite normal hemoglobin, bilirubin, and lactic dehydrogenase levels.

Case report

A 29-year-old primigravida in her thirty-seventh week of gestation came to the hospital with a 5-hour history of right upper quadrant pain. Blood pressure was 124/70 mm Hg. Her diastolic pressure had previously never exceeded 60 mm Hg. There was no peripheral edema. Laboratory investigations included: hemoglobin, 125 gm/L (normal, 120 to 160 gm/L); platelet count, 73×10^9/L (normal, 150 to 450 $\times$ 10^9/L); and normal values for white blood count, prothrombin time, activated partial thromboplastin time, and fibrinogen. Abnormal hepatic function tests included alkaline phosphatase, 200 U/L (normal, 25 to 100 U/L); alanine aminotransferase, 407 U/L (normal, 10 to 45 U/L); and aspartate aminotransferase, 296 U/L (normal 7 to 40 U/L). Serum bilirubin and lactic dehydrogenase levels were normal. A 24-hour collection of urine did not reveal proteinuria.

Red cell morphology was microscopically assessed in 30 high-power ($\times$ 1000) fields, and 1.3 schistocytes were noted per field. In five nonanemic, predelivery subjects assessed in the same laboratory, schistocyte counts averaged 0.3 per high-powered field. Serum haptoglobin level was found to be depressed at 0.6 gm/L (lower limit of normal 1.0 gm/L).

Within 2 days of admission, spontaneous delivery occurred. Normalization of the patient's platelet count occurred in 2 days and correction of the hepatic function test abnormalities and the serum haptoglobin was documented three weeks postpartum.

Comment

In Weinstein's[1] update of his experience with the HELLP syndrome, 48 of 56 patients had abnormal peripheral blood films, including the presence of burr cells and/or schistocytes. Sibai et al.[2] reviewed 112 patients, and all were found to have abnormal peripheral smears and lactic dehydrogenase levels above 600 IU/L. Neither commented on the degree of morphologic changes in the blood films of their patients, and Weinstein did not comment on evidence of hemolysis in the eight patients who had a normal peripheral film.

Our patient was not anemic and did not have an elevated bilirubin or lactic dehydrogenase level but had modest morphologic evidence of microangiopathic hemolysis. To corroborate the occurrence of intravascular hemolysis, the serum haptoglobin was assayed and found to be decreased. Haptoglobin is an α_2-plasma protein that binds to free hemoglobin and forms a complex that is removed by the reticuloendothelial system. A low level of haptoglobin is a sensitive indicator of intravascular red cell destruction. In this case, the lowered serum haptoglobin level was valuable in confirming the presence of hemolysis despite the absence of anemia, hyperbilirubinemia, or elevated lactic dehydrogenase levels.

In conclusion, in suspected cases of the HELLP syndrome in which evidence of hemolytic anemia is absent or modest, assay of the serum haptoglobin may assist in the diagnosis.

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Insufficient transplacental digoxin transfer in severe hydrops fetalis

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A case of severe nonimmune hydrops fetalis caused by supraventricular tachycardia is presented. Maternal treatment with digoxin and the subsequent addition of verapamil and propranolol failed to be effective. Simultaneous measurement of maternal serum and cord blood digoxin levels showed insufficient transplacental digoxin transfer. Other modalities of treatment are discussed. (Am J Obstet Gynecol 1987;157:1268-9.)

Key words: Nonimmune hydrops fetalis, supraventricular tachycardia, digoxin treatment

It has been suggested that aggressive transplacental therapy of fetal supraventricular tachycardia would be preferable to premature termination of pregnancy and subsequent extrauterine therapy, especially when the arrhythmia is associated with cardiac compromise and hydrops fetalis.[1]

Maternal digoxin administration remains the treatment of first choice in these cases, with the goal of attaining a maternal digoxin level at the upper end of the therapeutic range. It has been reported that the cord blood digoxin level in nonhydropic fetuses at labor was similar to the maternal level at birth in patients with cardiac disease treated with digoxin.[2]

Case report

We recently encountered a 34-year-old woman, gravida 4, para 2, in the twenty-seventh week of gestation whose fetus was severely hydropic as a result of supraventricular tachycardia of 260 bpm. A complete search for other causes of nonimmune hydrops fetalis was negative. Ultrasonic examination showed scalp edema, severe ascitis, severe pleural effusion, pericardial effusion, placentomegaly with a maximal thickness of 75 mm, and severe oligohydramnios. Echocardiography of the fetal heart anatomy was normal, and supraventricular tachycardia was clearly verified. Treatment was initiated with maternal digoxin administration (a loading dose of 1.25 mg by mouth and a maintenance dose of 0.425 to 0.5 mg/dl) with the subsequent addition of verapamil and propranolol, but sinus rhythm was not restored and the hydrops deteriorated. Maternal digoxin serum levels, which were measured by radioimmunoassay, were 1.1 to 1.9 ng/ml. Three weeks after the initiation of antiarrhythmic therapy, fetal membranes ruptured spontaneously and abruption of the placenta was diagnosed. A cesarean section was immediately performed, and a severely hydropic male fetus weighing 2175 gm was delivered. The placenta weighed 1350 gm and was extremely edematous. At operation the maternal ovaries were noted to be edematous and polycystic. Sinus rhythm (140 bpm) was restored spontaneously after the umbilical cord was clamped. The newborn infant was resuscitated and mechanically ventilated but after 3 days died of severe respiratory insufficiency. Simultaneous measurements of maternal and cord blood, ovarian follicular fluid, and fetal ascitic fluid for digoxin levels are shown in Table I. These figures indicate insufficient transplacental digoxin transport.

Table I. Digoxin levels in the biologic fluids of the treated mother and fetus with hydrops fetalis

	Biologic fluid	*Digoxin levels (ng/ml)*
Maternal	Serum	1.1
	Ovarian follicular fluid	1.1
Fetal	Cord blood serum	0.3
	Ascitic fluid	0.3

Comment

It seems that the apparent passive transfer of digoxin across the placenta in normal fetuses[2] was probably disturbed in our patient by the high intravascular hydrostatic pressure existing in cases of hydrops fetalis. As in other cases reported in the literature, treatment failure may also be the result of severe placental edema and increased maternofetal diffusion distance.

The prognosis of prematurely delivered fetuses with hydrops is very unfavorable.[1] Therefore in cases of severe hydrops resulting from fetal arrhythmia with associated extreme edema of the placenta that do not respond to maternal administration of antiarrhythmic

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drugs, other therapeutic measures should be considered. An attempt may be made to reverse the arrhythmia by compression of the umbilical cord, or else the drug may be injected directly into the umbilical cord or the fetal gluteus muscle by means of ultrasonic guidance. The possibility of maternal administration of an antiarrhythmic drug that actively cross the placenta, such as procainamide, may also be considered.

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Renal arterial blood flow velocity waveforms in pregnant women

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Seventy-two continuous-wave Doppler ultrasonographic examinations were performed on nine nonpregnant and 56 normal pregnant women. The range of gestation was 6 to 40 weeks, and the objective was to record the renal arterial blood flow velocity waveform. Acceleration time and systolic per diastolic ratio of renal arterial blood flow velocity waveforms were assessed. Of 72 examinations, 68 recordings were made of the left renal artery (94.4%) and 28 of the right renal artery (38.9%), respectively. There was no correlation between acceleration time and systolic per diastolic ratio and gestational age, respectively, and no correlation between acceleration time and systolic per diastolic ratio, and caliceal diameter of the renal pelvis, respectively. Acceleration times of the left and right renal arteries were 47 ± 11 and 40 ± 8 msec in nonpregnant women and 62 ± 23 and 62 ± 23 msec in pregnant women, respectively. The left and right systolic per diastolic ratios were 3.1 ± 0.7 and 2.9 ± 0.5 in nonpregnant women and 2.8 ± 0.6 and 2.6 ± 0.5 in pregnant women, respectively. Therefore these data provide a foundation for the study of hydronephrosis, which may occur in pregnant women. (Am J Obstet Gynecol 1987;157:1269-71.)

Key words: Doppler ultrasound, renal artery, hydronephrosis, pregnancy

Ultrasonographic assessments of asymptomatic maternal hydronephrosis in pregnancy have been reported.[1-4] Regression occurred gradually after delivery.[5] There are at least two theories regarding the etiology, namely, hormonal effect and mechanical compression.[6]

With recent advances in Doppler ultrasound examination, measurements of renal arterial blood flow velocity waveforms have been made to predict renal function and damage in normal, abnormal, and allografted kidneys.[7-9] To the best of our knowledge, there has been no documentation of maternal renal arterial blood flow velocity waveforms obtained by Doppler ultrasound examination in pregnant women. We have now completed continuous-wave Doppler ultrasonographic measurements of maternal renal arterial blood flow, and we assessed changes in renal blood flow in pregnant patients with hydronephrosis.

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Patients and methods

Seventy-two continuous-wave Doppler ultrasonographic examinations were performed on nine nonpregnant and 56 normal pregnant Japanese women who were seen in our clinics at Shimane Medical University Hospital. The range of gestation was 6 to 40 weeks. Permission was obtained from each woman for the study.

We used an Aloka SSD-730 with a 2 MHz transducer, and it is applicable for either pulsed or continuous-wave Doppler mode. With this apparatus, simultaneous real-time imaging and Doppler tracings can be made.

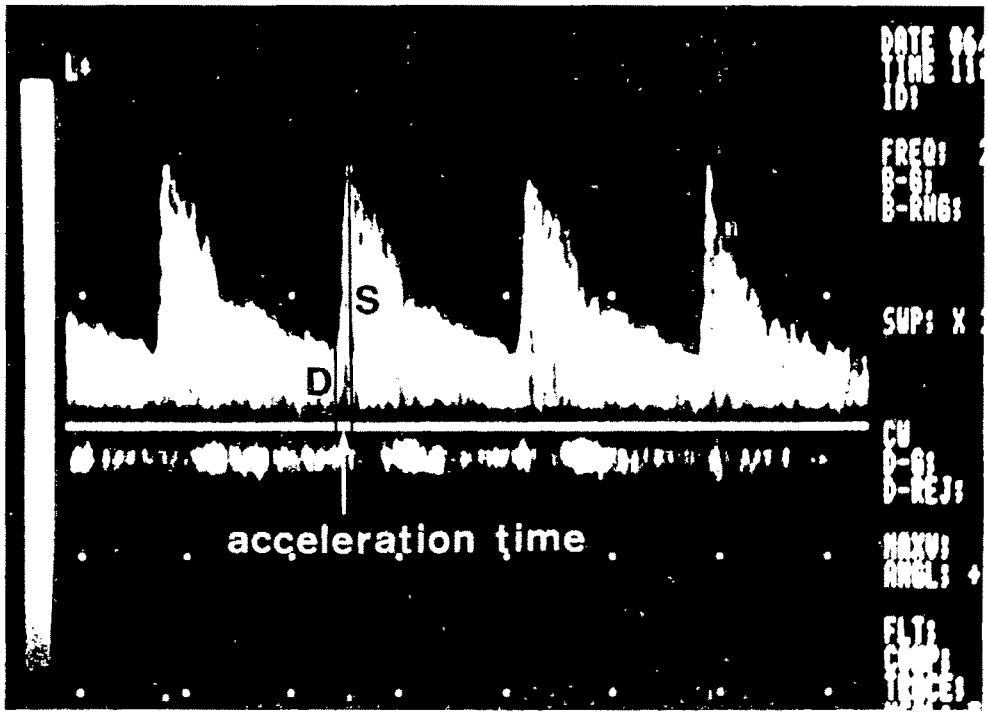


Fig. 1. Blood flow velocity waveforms of the left renal artery in a woman who was 31 weeks' pregnant. (*D* = Least diastolic flow velocity; *S* = peak systolic flow velocity.)

Table I. Rates of detection of renal arterial blood flow velocity waveform

	Gestational age (wk)	*Detected*	
		Left kidney (%)	*Right kidney (%)*
Nonpregnant (n = 9)		9 (100)	8 (88.9)
Pregnant	6-11 (n = 9)	9 (100)	3 (33.3)
	12-19 (n = 12)	11 (91.7)	2 (16.7)
	20-27 (n = 7)	7 (100)	0 (0)
	28-35 (n = 17)	14 (82.4)	9 (52.9)
	36-40 (n = 18)	18 (100)	6 (33.3)

Table II. Values of left and right renal arterial blood flow velocity waveforms in nonpregnant and pregnant women

	Acceleration time (msec)	*Systolic per diastolic ratio*
Nonpregnant		
Left kidney (n = 9)	47 ± 11	3.1 ± 0.7
Right kidney (n = 8)	40 ± 8	2.9 ± 0.5
Pregnant		
Left kidney (n = 59)	62 ± 23*	2.8 ± 0.6
Right kidney (n = 20)	62 ± 23*	2.6 ± 0.5

*$p < 0.05$.

In the continuous-wave Doppler mode, velocities can be measured up to 6 m/sec but with loss of range resolution.

The patient was placed in the left decubitus position, and the kidneys were scanned in the longitudinal plane from the back by real-time ultrasound examination. The most dilated calyx was identified and measured from the leading edge to leading edge with electronic calipers, and classification into four groups was made.[8] The continuous-wave Doppler beam was directed to the area of the renal hilus, and a search was made for strong pulsatile signals, which indicated a reflection of the beam from the renal artery. The renal arterial blood flow velocity waveform is shown in Fig. 1. Acceleration time was measured as the interval from the start of the systolic forward phase to the maximal peak velocity in systole. The systolic per diastolic ratio (the ratio of peak systolic to least diastolic flow velocity) was calculated. On each record, three to four separate cardiac cycles were examined to determine the mean acceleration time or systolic per diastolic ratio. Statistical analysis was performed by the Student *t* test.

Results

Of 72 examinations, 68 recordings were made of the left renal artery (94.4%) and 28 were made of the right renal artery (38.9%), respectively (Table I). Of these 79 (59 left and 20 right kidneys) in pregnant women,

54 were grade 0 (caliceal diameter 0 to 5 mm), 19 were grade I (caliceal diameter 6 to 10 mm), one was grade II (caliceal diameter 11 to 15 mm), and five were grade III (caliceal diameter ≥16 mm), respectively. On the other hand, grade 0 was 16 and grade I one of 17 (nine left and eight right kidneys) in nonpregnant women. There was no correlation between acceleration time and systolic per diastolic ratio and gestational age, respectively. There was also no correlation between acceleration time and systolic per diastolic ratio and the caliceal diameter. Acceleration times of the left and right renal arteries were 47 ± 11 and 40 ± 8 msec in nonpregnant women, and 62 ± 23 and 62 ± 23 msec in pregnant women, respectively (Table II).

Acceleration time in pregnant women was slightly longer than that in nonpregnant women ($p < 0.05$). The left and right systolic per diastolic ratios were 3.1 ± 0.7 and 2.9 ± 0.5 in nonpregnant women and 2.8 ± 0.6 and 2.6 ± 0.5 in pregnant women, respectively (Table II).

Comment

With advances in high-resolution ultrasonic equipment, an appropriate observation of cases of maternal hydronephrosis during pregnancy can be made.[1, 2] Fried[2] found that the onset of dilatation was earlier than that reported, the degree of hydronephrosis in the right kidney predominated, and that there was a gradual increase in the extent of dilatation throughout pregnancy. Peake et al.[3] used renal caliceal diameter as an index of hydronephrosis and noted a gradual increase in dilatation throughout pregnancy; the rate of increase was greater in the right than in the left kidney. In our study, 25 of 79 kidneys each (31.6%) in pregnant women showed some dilatation of the renal pelvis, and five with grade III hydronephrosis (severe dilatation, caliceal diameter ≥16 mm)[3] were right sided.

Sampson[7] was the first to measure renal arterial blood flow velocity waveform by Doppler ultrasound examination and to monitor the rejection process in human renal allotransplants. In the case of healthy subjects, the renal arterial blood flow pattern is composed of a fast systolic forward flow followed by a gradually slowing diastolic flow continuing to end-diastole.[9] On the other hand, a significant correlation was observed between the acceleration time of flow velocity waveform and graft function, and there was no correlation among systolic per diastolic ratio and graft function.[8] In healthy subjects, the acceleration time was 50 ± 7 msec, and the systolic per diastolic ratio was 3.4.[3] In the present study, there was no correlation between acceleration time and systolic per diastolic ratio and gestational age, respectively, and no correlation was found between acceleration time and systolic per diastolic ratio and caliceal diameter of the renal pelvis. Acceleration times of the left and right renal arteries were 47 ± 11 and 40 ± 8 msec in nonpregnant women and 62 ± 23 and 62 ± 23 msec in pregnant women, respectively. The left and right systolic per diastolic ratios were 3.1 ± 0.7 and 2.9 ± 0.5 in nonpregnant women, and 2.8 ± 0.6 and 2.6 ± 0.5 in pregnant women, respectively. Therefore these data provide a foundation for the study of hydronephrosis of pregnancy.

Kinoshita[9] reported that abnormalities of renal arterial blood flow velocity waveform were seen in cases of renal parenchymal damage, such as chronic glomerulonephritis and hydronephrosis. Therefore Doppler ultrasound examination is a pertinent tool when attempting to assess renal function and circulatory dynamics in cases of preeclampsia, toxemia of pregnancy, and other renal disease occurring in pregnant women.

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The endocrinologic evaluation of a 45,X true hermaphrodite

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A 13-year-old 45,X girl presented with spontaneous menses and the acute onset of progressive hirsutism. Complete endocrinologic evaluation of hirsutism was unremarkable. An exploratory laparotomy revealed a gonad containing ovarian and testicular tissue. Chromosome analysis from four different tissues confirmed the karyotype to be 45,X. She was H-Y antigen positive. (AM J OBSTET GYNECOL 1987;157:1272-3.)

Key words: True hermaphrodite, Turner's syndrome, hirsutism

A true hermaphrodite is an individual whose gonad(s) contain ovarian tissue with follicles and testicular tissue with spermatozoa or seminiferous tubules.[1] (Women with a 45,X chromosomal complement have either streak gonads containing ovarian stroma devoid of follicles or, rarely, present with normal ovarian tissue.) We present the first case of a 45,X true hermaphrodite. Diagnosis was made after the patient complained of the acute onset of progressive hirsutism.

Case report

This patient first presented to our clinic with the diagnosis of Turner's syndrome and was referred because of vaginal bleeding. A chromosomal analysis had been performed on peripheral lymphocytes in the neonatal period to establish a diagnosis. Starting at age 12 years 4 months, she described the spontaneous onset of vaginal bleeding. This occurred twice more over the next 6 months. Thelarche and pubarche preceded menstruation by approximately 8 months. The rest of her history was unremarkable. At physical examination of this 13-year-old girl (height = 54 inches; weight = 70 pounds), classical Turner's facies, T3 breasts, and P3 pubic hair were present. There was no evidence of androgen excess. External genitalia and vaginal mucosa demonstrated an estrogenic effect. Blood was noted to be coming from the cervical os. The uterus was normal and ovaries were not palpable. Laboratory evaluation revealed elevated gonadotropin levels (luteinizing hormone, 56 mIU/ml; follicle-stimulating hormone, 225 mIU/ml) with a low serum estradiol level (10 pg/ml) at the time of this menses. The assessment at the time was that she was having periodic follicular development.

She returned to the clinic 5 months later complaining of the rapid onset of hirsutism and acne. The Ferriman/Gallwey system was used to score the hirsutism. She was given a score of 14 (lip 3, chest 2, lower back 3, lower abdomen 2, forearm 2, and thigh 2) with normal being <4 and only 1.2% of women scoring >10. Hirsutism is not an associated finding in Turner's syndrome. A bimanual pelvic examination and an ultrasound examination failed to demonstrate a pelvic mass. Serum androgen levels, except for dehydroepiandrosterone sulfate, were within normal limits for a peripubertal woman (testosterone = 29 ng/ml; non–sex hormone binding globulin bound testosterone = 9%; androstenedione = <20 ng/dl; initial dehydroepiandrosterone sulfate = 3.73 and repeat 2.80 μg/ml). Adrenal status was evaluated by an adrenocorticotropic hormone stimulation test (17-hydroxy progesterone: before test, 1.91 ng/ml; after test, 2.08 ng/ml) and a computed tomographic scan of the adrenals, which was normal.

Patients with idiopathic hirsutism have elevated levels of 5α-reductase activity in the skin. In order to ascertain whether this patient had increased 5α-reductase activity and therefore increased end-organ sensitivity to testosterone, pubic skin was analyzed for 5α-reductase activity in vitro. She demonstrated a normal conversion of testosterone to dihydrotestosterone (1.64%/200 mg/hr; normal 1% to 5% per 200 mg per hour).

There was no reasonable explanation for the hirsutism so a laparotomy was performed to evaluate the gonads for a Y line. Uterus and fallopian tubes were normal; there was a streak gonad on the left and a small gonad measuring 2.3 by 1.5 by 1.3 cm on the right. Blood was taken from the ovarian veins bilaterally and was analyzed for testosterone and estradiol. A gradient was demonstrated between the right gonad and the periphery (right gonad: estradiol = 108 pg/ml, testosterone = 48.4 ng/dl; peripheral vein: estradiol = 50 pg/ml, testosterone = 19.4 ng/dl).

Pathologic examination of the streak gonad showed only fibrous tissue but the right gonad revealed a dysgenetic gonad with mature graafian follicles with ova, a marked decrease in the number of primordial follicles; Sertoli-Leydig cells, and well-developed seminiferous tubules. This established the diagnosis of true

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hermaphroditism. Chromosome analysis was performed on tissue from each gonad, skin, and lymphocytes. One hundred cells from each source were counted, and karyotypes of 10 from each were determined. All results confirmed the original karyotype of 45,X. The H-Y antigen status was positive and the postoperative estradiol, testosterone, and dehydroepiandrosterone sulfate levels were 16 pg/ml, <10 ng/ml, and 2.6 μg/ml, respectively.

Comment

We present here the first description of a 45,X true hermaphrodite. It is impossible to prove that mosaic lines do not exist, for example, a 46,XX line to explain menstrual function. However, this cell line could not be demonstrated even though karyotypes of the ovotestis and three other tissues were determined and a total of 400 cells were counted. The presence of the H-Y antigen is expected with the finding of testicular tissue. The mechanism of hirsutism in this patient remains unexplained because the androgen levels were never found to be sufficiently elevated and she had normal 5α-reductase activity. However, hirsutism has been reported previously in a patient with gonadal dysgenesis and low levels of androgens.[2] We concur that surgical evaluation of the gonads should be performed if no other explanation of hirsutism can be found to evaluate the possibility of a Y line in patients with a 45,X karyotype.

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Hyperimmunoglobulinemia E and pregnancy: A case report

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Hyperimmunoglobulinemia E is characterized by recurrent bacterial sinopulmonary and skin infections from birth or early childhood, with IgE levels at least 10 times greater than the upper limits of normal. The following case describes a young black woman with hyperimmunoglobulinemia E syndrome who had an uneventful pregnancy and delivery. The infant has been diagnosed as suffering from hyperimmunoglobulinemia E syndrome as well. (Am J Obstet Gynecol 1987;157:1273-4.)

Key words: Hyperimmunoglobulinemia E, pregnancy

A case is presented of a young black woman with hyperimmunoglobulinemia E syndrome.

Case report

P. D., a 21-year-old black woman, gravida 1, para 0, was seen in our prenatal clinic for the first time at 13 weeks' gestation. The patient had been followed up at the Children's Hospital of Philadelphia because of hyperimmunoglobulinemia E syndrome for more than 10 years. She has suffered from recurrent sinopulmonary infections with resultant chronic obstructive pulmonary disease, as well as recurrent staphylococcal skin infections with abscess formation requiring dicloxacillin and drainage.

The patient's family history includes a brother with hyperimmunoglobulinemia E and a sister who died from complications of hyperimmunoglobulinemia E.

Physical examination revealed a well-developed black woman with coarseness of facial features and scarring of the face and skin. She had onycholysis and mild clubbing of the distal phalanges. Examination of the lungs revealed scattered rhonchi. The pelvic examination revealed the uterus to be compatible with a 13-week pregnancy.

The laboratory studies revealed a white blood cell count of 6300/mm^3 with 50 neutrophils, 16 eosinophils, 26 lymphocytes, and eight monocytes. The serum IgE level was >10,000 IU/ml (n = 195) and the antistaphylococcal IgE was 13.5 (n = 0).

The pregnancy progressed uneventfully until the twenty-seventh week of gestation, when the patient was admitted with an exacerbation of bronchitis and de-

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hydration. The dehydration was secondary to diarrhea. Stool examination revealed giardiasis. The patient was treated with nafcillin and furazolidone.

Beginning at 28 weeks, the patient underwent serial ultrasound examination for evaluation of fetal growth.

The patient was readmitted at 32 weeks' gestation with productive cough and weight loss. The sputum culture revealed *Pseudomonas* and *Proteus* bacteria. The patient was treated with cefaclor. She was also noted to have a gingival abscess that required surgical drainage.

The patient had no further antepartum complications and went into spontaneous labor on October 18, 1986, at 40 weeks' gestation. She was delivered of a healthy baby girl weighing 6 pounds 7 ounces. The postpartum period was uneventful. At a follow-up postpartum visit the patient was doing well. Her infant has recently been diagnosed as having hyperimmunoglobulinemia E syndrome and is taking oral dicloxacillin and steroid skin cream.

Comment

In 1966, Davis et al[1] described two female patients with fair skin, reddish hair, severe eczema, dystrophic fingernail changes, severe sinopulmonary infections, and recurrent staphylococcal abscesses. The abscesses did not show any surrounding erythema and were termed "cold abscesses." The newly perceived syndrome was called Job's syndrome from the biblical quotation. Subsequent work by others[2] has revealed that the syndrome is not limited to red-haired women, as it has been described in men and black persons.

Patients with hyperimmunoglobulinemia E syndrome have IgE levels at least 10 times greater than normal and a chemotactic defect in circulating neutrophils.[3] The patients usually have coarse facial features with a broad nasal bridge, a prominent nose, and irregularly prepositioned cheeks and jaws. *Candida* infections of the nails, mouth, and vagina are common. A familial occurrence has been observed without any definite inheritance pattern.

There have been no reported cases in the literature of pregnancy in these patients. The effect of hyperimmunoglobulinemia E syndrome on pregnancy is not known because this is a rare condition. Our patient was delivered of a healthy newborn infant at term.

We recommend that patients with hyperimmunoglobulinemia E syndrome undergo regular surveillance for detection of signs of infection. This will permit timely diagnosis and prevent sepsis. Pulmonary complications should be managed promptly and aggressively to prevent hypoxia. Fetal ultrasound examination should be done in the third trimester to assess fetal growth in the face of chronic obstructive pulmonary disease. The neonate should be screened for hyperimmunoglobulinemia E, as a familial inheritance can occur, as happened in this case.

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2-Chloroprocaine for local perineal infiltration

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Lidocaine was recently found to rapidly cross the placenta and result in considerable fetal exposure after local infiltration of the perineum for episiotomy. For this reason, a more appropriate agent for local perineal infiltration might be a drug with rapid metabolism and inactive metabolites. 2-Chloroprocaine, an ester-linked local anesthetic agent, is available but is not commonly used for this procedure. The purpose of this study was to evaluate the placental transfer of 2-chloroprocaine after local perineal infiltration. The drug was administered to 17 normal term pregnant women for episiotomy. After local perineal infiltration, the levels of 2-chloroprocaine and/or its metabolite, chloroaminobenzoic acid, were quantitated in maternal and neonatal plasma or urine. The pharmacologic data indicated that 2-chloroprocaine was nondetectable at delivery in maternal and umbilical cord vein; one patient had trace levels in the cord. Chloroprocaine was not detectable in neonatal plasma, but chloroaminobenzoic acid was detectable in both maternal and cord vein plasma. Mean levels of chloroaminobenzoic acid in maternal plasma at delivery were 1.04 ± 0.32 μg/ml, and mean levels in cord vein were 0.35 ± 0.54 μg/ml. Clinically, 2-chloroprocaine provided good analgesia. These results indicate that very little, if any pharmacologically active drug, reaches the fetus after local perineal infiltration with 2-chloroprocaine. Therefore 2-chloroprocaine appears to be preferable to lidocaine when used for local perineal infiltration. (Am J Obstet Gynecol 1987;157:1275-8.)

Key words: 2-Chloroprocaine, perineal infiltration, chloroaminobenzoic acid

Local infiltration of the perineum is a commonly performed procedure used to provide analgesia for episiotomy. The most commonly used local anesthetic agent for this purpose has been lidocaine. Since small quantities of lidocaine were used and administration occurred very close to delivery, it was assumed that very little drug would reach the fetus. However, in a recent study, lidocaine was found to rapidly cross the placenta with considerable drug levels in the umbilical cord vein at delivery.[1] Furthermore, the rapid placental transfer of lidocaine resulted in a mean fetal-maternal ratio significantly higher than that found with much larger doses of lidocaine for lumbar epidural anesthesia.

Thus lidocaine crosses the placenta in considerable amounts after local perineal infiltration. Furthermore, lidocaine is metabolized slowly in the liver into two pharmacologically active metabolites. Therefore other local anesthetic agents may be more appropriate for local perineal infiltration. One possibility is 2-chloroprocaine, an ester-linked local anesthetic agent that is rapidly metabolized by plasma enzymes into inactive metabolites.

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The purpose of this study was to determine (1) the disposition of 2-chloroprocaine and its metabolite, 2-chloroaminobenzoic acid, in mother, fetus, and neonate after local perineal infiltration and (2) if 2-chloroprocaine provides adequate pain relief.

Material and methods

Patients. Seventeen normal gravida at term from the obstetric service at Cleveland Metropolitan General/Highland View Hospital were studied. Informed consent for sample collection was obtained in early labor. Local infiltration of the perineum with 1% 2-chloroprocaine was administered as needed for episiotomy. No patients had a history of liver or kidney disease or of known drug abuse.

Procedure. Local perineal infiltration was performed with a 22-gauge needle. 2-Chloroprocaine without epinephrine was injected superficially into the area of the posterior fourchette during the crowning phase of the second stage of labor. The drug was injected as needed, and the amount injected was determined by the physician. The total dose of 2-chloroprocaine (milligrams) and the time (minutes) from administration to delivery (the drug-to-delivery interval) were recorded.

Sample collection. Maternal blood samples (3 ml) were collected from an indwelling cannula in a peripheral arm vein. After a premedication blood sample was obtained, blood samples were collected after local perineal infiltration at 1, 3, 5, 7, 10, 20, and 30 minutes or until delivery, at which time the final sample was drawn. Postpartum maternal blood samples were

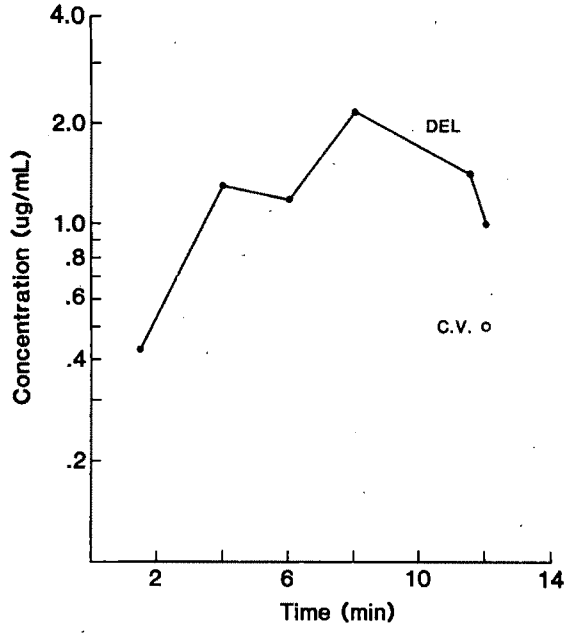


Fig. 1. Maternal plasma concentration of chloroaminobenzoic acid after local perineal infiltration with 8 ml of 1% 2-chloroprocaine (80 mg). (*C.V.* = Cord vein; *DEL* = delivery.)

Table I. Maternal, neonatal, and intrapartum anesthesia of the study group (N = 17)

Mothers		
Age (yr)*	20.0 ± 4.5	(16-27)
Parity		
Nulliparous	15	
Multiparous	2	
Neonates		
Gestational age (wk)	39.6 ± 1.2	(38-42)
Birth weight (gm)	3312 ± 411	(2660-4190)
Apgar score < 7		
1 min	4	
5 min	0	
Sex		
Male	11	
Female	6	
Intrapartum anesthesia		
Total dose (mg)	81.8 ± 27.0	(20-100)
Drug-to-delivery interval (min)	6.7 ± 4.3	(2-17)

*Mean ± standard deviation with range in parentheses.

obtained at 24, 48, and 72 hours after delivery. All samples were immediately put into heparinized Vacutainers and placed on ice. Samples to be analyzed for 2-chloroprocaine and chloroaminobenzoic acid levels were collected in Vacutainers containing 0.3 ml of a cholinesterase inhibitor, echothiophate iodide, 0.2 gm/ml (Ayerst Laboratories, New York, New York); samples analyzed for cholinesterase activity were obtained in Vacutainers without the inhibitor. The plasma was removed after centrifugation and frozen until assayed.

Neonatal blood samples were obtained at birth from a doubly clamped section of umbilical cord and at 24, 48, and 72 hours of age by either heel stick or venipuncture. Neonatal urine samples were collected in plastic urine collection bags (Hollister Newborn U-Bag, Libertyville, Illinois). Collection of six consecutive 6-hour samples was attempted, but collections were stopped if signs of skin irritation occurred. All urine samples were frozen until analyzed.

Specimen analysis. Plasma levels of 2-chloroprocaine were analyzed by single ion-monitoring gas chromatography and mass spectrometry.[2] The sensitivity of this method is 1 ng/ml of plasma. Plasma and urine levels of chloroaminobenzoic acid were analyzed by gas chromatography with a modification of a previously reported method.[3] The modifications included use of an electron capture detector for increased specificity and use of diiodoaminobenzoic acid as an internal standard. The sensitivity of this method is 50 ng/ml. Total chloroaminobenzoic acid in neonatal urine was analyzed. No attempt was made to distinguish between free and bound chloroaminobenzoic acid since only trace levels of conjugated chloroaminobenzoic acid have been reported in neonates.[4] Urinary chloroprocaine levels were not determined. Plasma cholinesterase activity and the dibucaine number were determined by spectrophotometry for documentation of normal cholinesterase levels.[5]

Pharmacokinetic analysis. Fetal-maternal ratios were calculated by dividing the amount of chloroaminobenzoic acid in the cord vein at birth by the amount in the maternal vein at birth. Disappearance of chloroaminobenzoic acid from urine was monitored by recording drug concentration in micrograms of drug excreted per milliliter against hours after delivery for the neonate.

Clinical evaluation. The quality and duration of pain relief were evaluated subjectively by each patient. If additional pain relief was required because of an extension of the episiotomy or a laceration of the vaginal mucosa, 1% lidocaine was administered.

Newborn evaluation consisted of 1- and 5-minute Apgar scores and fetal acid-base status from the umbilical cord vein. Each infant was weighed postpartum and underwent a complete physical examination, and gestational age was determined by the Ballard modification of the Dubowitz examination.[6]

Results

Patient population. The maternal, neonatal, and intrapartum anesthesia characteristics of the 17 study patients are shown in Table I. The mothers were at term and without any underlying obstetric problems. All neonates had Apgar scores of >7 at 5 minutes. Four

neonates had 1-minute Apgar scores <7. Three of these neonates had thick meconium at delivery and required endotracheal suctioning immediately. The other neonate had a shoulder dystocia. The mean pH of the umbilical cord blood at delivery was 7.32 ± 0.05. There were no signs of anesthetic toxicity in any of the neonates.

Plasma drug levels. 2-Chloroprocaine was not detectable in maternal plasma after local perineal infiltration or at delivery. Detectable levels of 2-chloroprocaine were found at delivery in only one umbilical cord vein sample. The concentration in this one umbilical cord was 4.84 ng/ml. 2-Chloroprocaine was not detectable postpartum in neonatal plasma.

The appearance of chloroaminobenzoic acid was rapid and detectable in all patients before delivery. An example of a maternal plasma concentration time profile is shown in Fig. 1. As seen in this figure, the highest maternal plasma level of chloroaminobenzoic acid (2.13 μg/ml) occurred 8 minutes after receiving 8 ml of 1% 2-chloroprocaine (80 mg).

The mean, standard deviation, and range of chloroaminobenzoic acid concentration at delivery in the maternal plasma and umbilical cord vein are shown in Table II. The means were 0.62 and 0.28 μg/ml, respectively. The fetal-maternal ratio of chloroaminobenzoic acid at delivery ranged from 0.32 to 1.39, with a mean of 0.80.

Urine drug levels. Thirty-one samples were collected from nine neonates. Seven of the nine neonates had measurable levels of chloroaminobenzoic acid in their urine. Half of the samples had levels of chloroaminobenzoic acid below the detection limit (50 ng). The concentrations ranged from 0 to 2.4 μg/ml. Urinary chloroaminobenzoic acid was detectable in some neonates 36 hours post partum.

Clinical evaluation. The quality and duration of pain relief as judged by each patient were adequate. There were no adverse effects. The quality and duration of analgesia as evaluated by the obstetrician were sufficient except in two patients in whom additional local anesthetic was necessary for fourth-degree episiorrhaphies.

Comment

The purpose of this study was to describe the disposition of 2-chloroprocaine and its metabolite, chloroaminobenzoic acid, in mother and neonate after local perineal infiltration. The key finding of this study indicates that very little, if any, unchanged 2-chloroprocaine reaches the fetus after local infiltration of the perineum with 2-chloroprocaine.

These results are similar to those from previous studies of 2-chloroprocaine administered by other routes. 2-Chloroprocaine has been shown to reach the fetus in only small amounts after paracervical block and lumbar epidural anesthesia.[3, 7] This apparent lack of placental transfer occurs because 2-chloroprocaine is rapidly metabolized by maternal plasma cholinesterases. This rapid metabolism was evidenced immediately by detectable chloroaminobenzoic acid levels and nondetectable levels of 2-chloroprocaine. In the present study, trace levels of 2-chloroprocaine were found in only one umbilical cord vein sample at delivery. Any unchanged 2-chloroprocaine that crossed the placenta was probably metabolized by fetal plasma cholinesterases.[8, 9] Although fetal levels in utero were not measured, these data suggest that very little active drug reaches the fetus after local perineal infiltration with 2-chloroprocaine.

Table II. Chloroaminobenzoic acid concentration (micrograms per milliliter) at delivery in maternal plasma and umbilical cord vein after local perineal infiltration

Sample	*Concentrations*	
Maternal plasma (at delivery)*	0.62 ± 0.38	(0.36-1.31)
Umbilical cord vein	0.28 ± 0.42	(0-1.43)
Fetal/maternal ratio†	0.80 ± 0.47	(0.32-1.39)

*Mean ± standard deviation with range in parentheses.
†Ratio of level in cord vein to level in maternal vein at delivery (mean of individual ratios and not ratio of means).

The neonatal excretion of chloroaminobenzoic acid has been reported after administration of 2-chloroprocaine for paracervical block and lumbar epidural anesthesia.[3, 4, 7] These reports consistently indicate that the neonate excretes less than 0.25% of the total maternal dose as chloroaminobenzoic acid. These findings support the premise that very little active drug reaches the fetus after local perineal infiltration with 2-chloroprocaine.

The fetal-maternal ratio of chloroaminobenzoic acid (0.80) is similar to the fetal-maternal ratio reported for lumbar epidural anesthesia for cesarean section and for paracervical block.[3, 4, 7] The clinical data revealed no adverse effects of 2-chloroprocaine or chloroaminobenzoic acid on the mother, fetus, or neonate. More important, 2-chloroprocaine provided adequate analgesia for the episiorrhaphy except in two patients who required additional local anesthetic for a fourth-degree episiorrhaphy.

What clinical significance, if any, do the results of this study have? A previous study indicated that a common, obstetric procedure traditionally performed with lidocaine can result in considerable neonatal drug exposure.[1] In fact, neonatal lidocaine toxicity with convulsions has been reported after accidental scalp injection with lidocaine at the time of episiotomy.[10] This study suggests that this exposure can be minimized

with the use of an equally effective, alternative, pharmacologic agent that is metabolized differently. Hence 2-chloroprocaine, an ester-linked local anesthetic agent, may be preferable to lidocaine for local perineal infiltration.

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Breast galactography in the treatment of infected cysts

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Galactography is a valuable procedure to localize the cause of nipple duct secretions or hemorrhage. A unique variant is illustrated by cannulating a periareolar sinus tract, which drains from an infected epidermoid cyst communicating with several ducts. (Am J Obstet Gynecol 1987;157:1278-80.)

Key words: Galactography, mammography, breast, cyst, fistula

Among examinations for the evaluation of abnormal breast discharge, routine mammography is generally ordered for initial screening. However, its false-negative rate is high, such that only half of malignant tumors are typically identified on routine films.[1] Galactography yields a more detailed image of ductal disease on injection of contrast medium into the affected nipple duct. We report for the first time a galactogram variant in which a draining fistula is cannulated outside the areolar zone to demonstrate an inflamed epidermal cyst.

Method

After a scout mammogram is taken, the breast drainage site is cleaned, and the draining duct or sinus tract is gently cannulated with a 27-gauge blunt needle. Radiopaque contrast medium is slowly injected until the patient complains of discomfort or until it leaks around the needle. If the gynecologist anticipates interventional therapy, methylene blue dye can also be injected to delineate the extent of the inflammatory or neoplastic process. Mammography films are then taken at the end of the procedure.

Case report

A 24-year-old white woman with fibrocystic disease had pain and swelling in her upper right breast, in addition to purulent drainage from a nipple duct. Six months earlier, she had an infected cyst lanced and drained within this area, but soon afterward the nipple discharge recurred and a sinus tract formed and drained along the supraareolar ridge.

Mammograms revealed extensive dysplasia but no dominant mass or skin thickening. A galactogram was performed next, but no fluid could be expressed from the nipple, so the draining sinus tract rather than the occluded duct was cannulated (Fig. 1). The contrast

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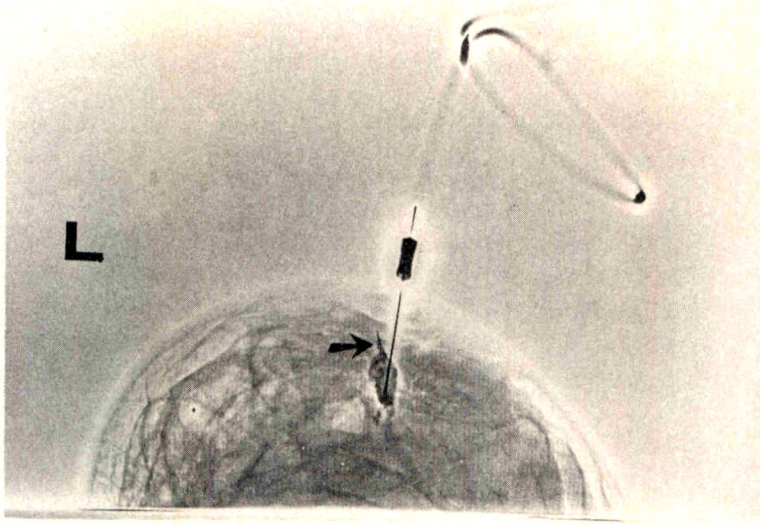


Fig. 1. Injection of contrast medium via the sinus tract into the cavity and partially occluded nipple duct *(arrow)*.

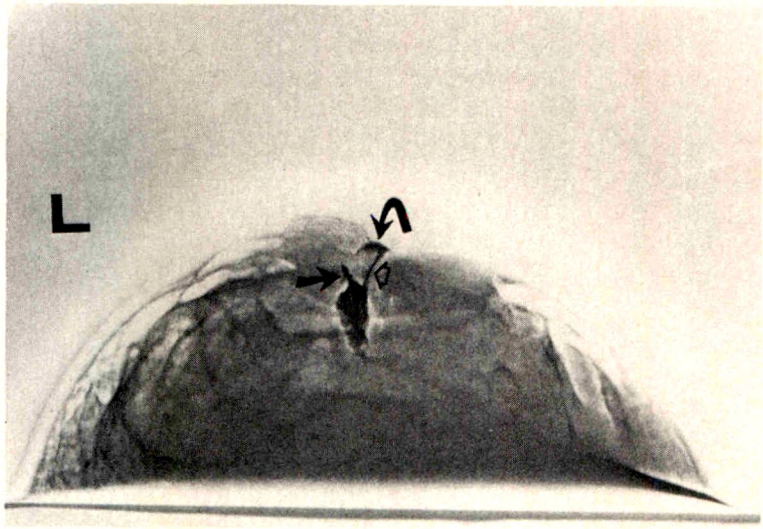


Fig. 2. Contrast opacification of the cavity with the sinus tract *(open arrow)*, partially occluded nipple duct *(straight arrow)*, and leakage outside the injection site *(curved arrow)*.

medium filled a small, irregular cavity connected with two occluded ducts, one of which extended deep into the breast and the other toward the nipple. On removal of the cannula, a final mammogram (Fig. 2) outlined the short sinus tract and internal communicating structures.

Examination of the biopsy specimen of the cavitary wall showed that it was an infected epidermoid cyst, which was derived from the epithelium around a hair follicle. After methylene blue dye was injected into the cyst, tissue resection was confined to the inflamed area. Major lumpectomy was thus avoided with good cosmetic results.

Comment

From its earliest use more than half a century ago,[2] galactography remains a key procedure for the evaluation of breast ductal diseases. Galactograms may delineate abnormalities such as filling defects, ductal caliber changes, amputation, or partial occlusion, and splaying or displacement of ducts. When properly performed, this procedure is more accurate and specific than the alternatives, including mammography, thermography, ultrasonography, transillumination, or clinical palpation.

There is no contraindication to its use, although be-

fore the introduction of water-soluble contrast medium, sequelae of pain or inflammation were cited after oily injections.[2] Among causes of serous or bloody discharge, papillomas are most frequently reported with an incidence of 40% compared to carcinoma with a lower incidence of 10%.[1] Thus galactography is an optimal technique for the diagnosis and delineation of duct- or even fistula-related diseases of the breast.

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Effects of the antiprogesterone RU 486 on preterm birth in the rat

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The effects of the antiprogesterone compound RU 486 on preterm delivery, myometrial gap junctions, and plasma levels of steroid hormones were investigated in pregnant rats. Injection of RU 486 on day 16 of gestation resulted in prolonged delivery beginning after 24 hours. The preterm birth initiated by RU 486 was preceded and accompanied by the extensive development of gap junctions between myometrial cells. R 5020, but neither progesterone nor dexamethasone, prevented preterm birth and the development of gap junctions. Estrogen and progesterone levels declined after RU 486 treatment but only after a major proportion of the fetuses were delivered. This study indicates that progesterone may normally inhibit uterine contractility by suppressing the genome responsible for the expression of gap junctions. (Am J Obstet Gynecol 1987;157:1281-5.)

Key words: Progesterone, labor, gap junctions, pregnancy, myometrium

The antiprogesterone compound RU 486 binds with high affinity to the progesterone receptor to inhibit the action of the hormone.[1] In some species, such as rats, rabbits, and sheep, elevated uterine levels of progesterone are thought to be required for the maintenance of pregnancy, and the decline in the hormone is believed to initiate labor.[2] The events that initiate human labor have never been adequately documented, and the role of progesterone has been the subject of much controversy. However, in all species studied including humans, treatment with RU 486 either prevents implantation, if given early or initiates parturition when given late in gestation,[1] indicating the importance of progesterone in maintaining pregnancy in all animals. The mechanism by which progesterone and the antihormone act to respectively sustain pregnancy and prevent or interrupt it is not understood. It is known that the withdrawal of progesterone stimulates the development of gap junctions between myometrial cells by estrogen-dependent mechanisms in rodents.[3, 4] Furthermore, these cell-to-cell contacts seem essential for the coordination of myometrial contractility in all species since they are present only in large numbers during term and preterm labor in humans and other species studied.[3-4] The presence of gap junctions appears to explain how hormonal regulation is translated into structural changes in the myometrium, which lead to conditions favoring contractility.[4] However, exactly how hormones control the development and regression of myometrial gap junctions is not completely understood. It has been suggested that either changes in steroid hormone and prostaglandin levels and/or changes in their receptors may govern the synthesis of gap junction proteins.[1-6]

In this study we treated rats at day 16 of gestation with a single injection of RU 486 and thereafter measured delivery of the fetuses, plasma levels of progesterone and estradiol, and gap junctions. Our study shows that RU 486 induced preterm delivery starting 24 hours after treatment. Fetal delivery was preceded and accompanied by the appearance of large numbers of gap junctions between the uterine muscle cells. We observed no changes in peripheral blood levels of the hormones until after 80% of the fetuses were born. These results suggest that progesterone may control the maintenance of pregnancy by reacting with its specific receptor directly in the myometrial cells to control the expression of gap junctions.

Material and methods

Pregnant rats (Sprague-Dawley strain) were injected subcutaneously with either sesame oil (controls) or sesame oil with RU 486 (10 mg/kg; Roussel Uclaf) on the morning of the sixteenth day of gestation (day 1 = sperm positive). Some RU 486-treated rats were also injected twice daily with either dexamethasone (100 μg/rat), progesterone (10 mg/rat), or R 5020

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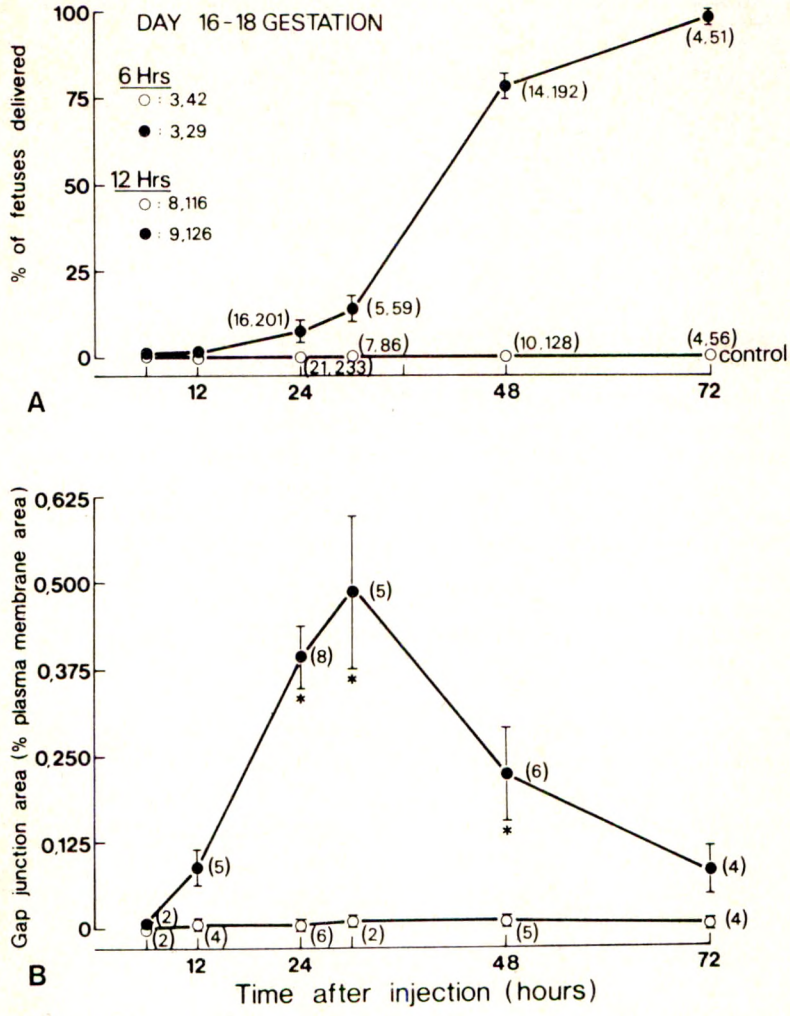


Fig. 1. Changes in delivery of fetuses **(A)** in control *(open circles)* and RU 486-treated *(closed circles)* pregnant rats. (Points represent means of percentage of total fetuses delivered ± SEM.) Numbers in brackets indicate the total number of pregnant rats and fetuses **(A)** and the number of tissues examined under the electron microscope **(B)** in each group at various times after treatment. *(Asterisk* = Mean values with significant differences [$p < 0.05$].)

(5 mg/rat). At various times some animals were killed (total pregnant rats = 144), and the number of fetuses in utero and placental attachment sites (total fetuses = 1747) were counted. Blood was collected in heparinized syringes for analysis of the steroid hormones. The midsection of one uterine horn was fixed for electron microscopy examination. Estradiol and progesterone were later assayed by radioimmunoassay. Tissues for electron microscopy were prepared as described previously.[5] The area of gap junction was determined from measurements of the lengths of junctional and nonjunctional membranes in 22 electron micrographs taken of the longitudinal muscle layers of each tissue (1 tissue/rat). Levels of significance were determined by an analysis of variance for multiple comparisons and the Student *t* test for pairwise comparisons ($p < 0.05$ = level of significant differences).

Results

Fig. 1, *A* shows the mean percentage of fetuses delivered (number of deliveries/total fetuses/rat for all rats in each group ± SEM) in control and RU 486-treated rats from 6 to 72 hours after injection. There were no obvious deliveries in control rats at any time despite the observation that some fetuses failed to develop (about 2%). There were also no deliveries in the RU 486-treated animals after 6 and 12 hours of treatment. At 24 hours after RU 486 injection, most rats had vaginal bleeding, and about 10% of the fetuses had been born. Between 30 and 48 hours, the greatest number of fetuses were delivered (about 70%). Thereafter to 72 hours, all remaining fetuses were expelled (about 20%). Treatment of other RU 486-injected rats with either dexamethasone (n = 13) or progesterone (n = 10) failed to significantly alter the delivery pattern



Fig. 2. Electron micrograph of myometrial tissue from a rat that delivered after 30 hours treatment with RU 486. (Original magnification ×146,000.)

as seen with RU 486 alone. However, treatment of animals with RU 486 and R 5020 (n = 16) completely prevented the preterm delivery produced with RU 486 alone (data not shown). No maternal deaths occurred after any of the treatments. Fetal deaths in utero were not apparent before 48 hours after RU 486 treatment but were present in about half the fetuses remaining (i.e., about 10% total) after 48 hours. None of the fetuses survived after birth, which was probably attributable to their prematurity.

The mean area of gap junction membrane (percentage of plasma membrane area ± SEM) between muscles cells in myometrial tissues from control and RU 486-treated rats is shown in Fig. 1, *B*. Only a few small gap junctions were found in tissues from control animals at all times. In contrast, tissues from animals that received RU 486 contained significantly higher ($p < 0.05$) gap junction areas at 24, 30, and 48 hours after treatment when the greatest percentage of fetuses were delivered (Fig. 1, *B* compared with Fig. 1, *A*). Gap junction areas were low (not significantly different from control tissue) at 72 hours after delivery of all fetuses. Similarly, gap junction areas were high in the delivery of rats treated with either RU 486 and dexamethasone (percentage area = 0.305 ± 0.070 at 24 hours, n = 4, and 0.180 ± 0.092 at 48 hours, n = 4) or RU 486 and progesterone (percentage area = 0.278 ± 0.045 at 24 hours, n = 4 and 0.012 ± 0.008 at 48 hours, n = 4) but were not different than control values in animals that received RU 486 and R 5020 (percentage area = 0.008 ± 0.008 at 24 hours, n = 4, and 0.005 ± 0.005 at 48 hours, n = 4) that did not undergo preterm delivery.

An electron micrograph of a gap junction between muscle cells of the myometrium 30 hours after RU 486 treatment is shown in Fig. 2. The average length of the junctions was larger ($p < 0.05$; 208 ± 8 nm) in animals undergoing preterm delivery induced with RU 486 compared with control tissues (101 ± 15 nm), but the increase in area (Fig. 1, *B*) was mainly caused by the increase in the number of junctions (control = seven junctions in photographs of 21 tissues; RU 486 = 210 junctions in photos of 30 tissues).

There were no significant differences ($p < 0.05$) in plasma levels of progesterone and estradiol between control and RU 486-treated rats until 48 hours (Table I) when 80% of the fetuses were already expelled (Fig. 1, *A*) and when high values of gap junctions were present (Fig. 1, *B*). At 48 hours, progesterone was significantly lower ($p < 0.05$) in maternal plasma of all groups treated with RU 486 except those also injected with R 5020 compared with control rats. Estradiol was also significantly lower at this time in all groups treated with RU 486, including those receiving R 5020. There were no significant differences in either progesterone or estradiol between control groups and those receiving dexamethasone alone at 24 and 48 hours.

Comment

This study shows that the antiprogestin RU 486 is efficient in producing an interruption of pregnancy as noted previously for rats[7] and primates,[8] including humans.[1, 9, 10] The results suggest that the progesterone receptor is involved in the action of progesterone to inhibit labor and support pregnancy. Furthermore, these results support the hypothesis that one of the

Table I. Plasma levels of progesterone and estradiol during later gestation in control and RU 486-treated rats

Treatment	*n*	*Progesterone (mg/ml)*	*Estradiol (pg/ml)*
Day 16, 12 hr			
Control	5	101 ± 14	74 ± 8
RU 486	6	113 ± 17	90 ± 7
Day 17, 24 hr			
Control	9	115 ± 11	71 ± 16
RU 486	13	126 ± 7	78 ± 9
DEX	4	115 ± 5	58 ± 7
DEX + RU 486	4	101 ± 9	66 ± 2
R 5020 + RU 486	3	146 ± 11	13 ± 3*
Progesterone + RU 486	3	199 ± 22*	99 ± 8
Day 17, 30 hr			
Control	3	138 ± 14	70 ± 6
RU 486	3	144 ± 6	69 ± 3
Day 18, 48 hr			
Control	7	124 ± 10	95 ± 22
RU 486	10	40 ± 8*	45 ± 11*
DEX	3	102 ± 9	88 ± 12
DEX + RU 486	3	34 ± 21*	66 ± 6
R 5020 + RU 486	4	126 ± 4	42 ± 15*
Progesterone + RU 486	7	158 ± 11*	15 ± 6*
Day 19, 72 hr			
Control	3	153 ± 3	114 ± 4
RU 486	4	11 ± 6*	85 ± 28

n = Number of rats in each group; DEX = dexamethasone-treated, 100 μg/rat/twice daily; Progesterone = 10 mg/rat/twice daily; R 5020 = 5 mg/rat/twice daily. RU 486 was given once at 10 mg/kg body weight.

*Significant differences ($p < 0.05$) between control and treated groups.

effects of progesterone, to maintain pregnancy, may be the selective repression of the genome that regulates the expression of gap junctions between myometrial cells.[4]

There is considerable evidence that RU 486 binds to the progesterone receptor with an affinity superior to progesterone and prevents the action of the hormone at the genomic level.[1] The progesterone antagonist prevents the transcription of several progesterone-dependent proteins in some tissues.[1, 11] In this study, however, the antihormone appears to block the suppressive effects of progesterone by inhibiting the action of it at the receptor level. The antihormone's effects were not mediated through changes in plasma levels of either progesterone or estradiol, which are thought to reflect tissue concentrations, since there were no alterations in these before delivery (Table I). The decline in progesterone and estradiol at 48 hours is probably a consequence of decreased synthesis as a result of delivery.

The mechanism for the maintenance of uterine quiescence during pregnancy and the initiation of labor contractions at term in any species has never been clearly established. It is recognized that either a change in progesterone, changes in progesterone to estrogen ratios, or changes in their receptors may trigger events leading to labor in many species.[2, 5, 6, 12] The fact that RU 486 effectively stimulates labor in all species including humans[1] supports the hypothesis[2] that progesterone plays a role in the maintenance of pregnancy in all species.

Gap junctions are present between myometrial cells in large numbers during term and preterm labor in rats, humans, and other species.[3, 5] Gap junctions are membrane proteins that provide channels between the interior of cells.[13] Their presence in the myometrium is accompanied by and thought to be necessary for the synchronized and coordinated contractions of labor when the muscle cells are better coupled electrically and metabolically.[14, 15] There is much evidence to suggest that myometrial gap junction proteins are the product of a genome that is stimulated by estrogen and inhibited by progesterone.[3, 5, 6, 16] In addition, studies show that steroid hormones may similarly control the synthesis of receptors for uterine stimulants.[17] The results of the present study support these conclusions and demonstrate that progesterone and its receptors may regulate (i.e., inhibit) the synthesis of specific gene products required for labor.

The gap junction areas (Fig. 1, *B*) found in this study after RU 486 treatment are higher (at 24 and 30 but not at 48 hours) than those normally observed in the rat myometrium during both spontaneous delivery at term[4, 5, 15] and preterm delivery after ovariectomy.[18] Thus an inhibition of progesterone action appears to have greater efficacy than gradual withdrawal of the hormone. However, the delivery of the fetuses is more prolonged (Fig. 1, *A*) than observed at term, and this may indicate that the uterus has not yet developed sufficiently or that there is an inadequate stimulus for contractility. In this regard, it should be noted that RU 486 treatment in combination with uterine stimulants increase the success rate of delivery in women.[1] Perhaps after RU 486 treatment, the myometrium is prepared for labor with gap junctions and appropriate receptors for stimulants but lacks the necessary stimulation provided by oxytocin or excitatory prostaglandins.

The increase in gap junction area precedes the delivery of the fetuses (Fig. 1, *A* and *B*), and gap junction values are highest during periods (at 24 and 30 hours) when the greatest proportion of fetuses (about 70%) are delivered. These data are similar to results obtained during spontaneous delivery at term in rats.[3, 4] The mean area of gap junctions noted at 48 hours is lower than that found at 24 and 36 hours but still as high as that during normal term parturition in rats.[4]

RU 486 also binds to the glucocorticoid receptor and produces antiglucocorticoid action in high doses.[1] However, our study shows that dexamethasone has no effect

on either the RU 486-induced delivery or the development of gap junctions. These results suggest that the antiglucocorticoid effect of RU 486 is not important for the interruption of pregnancy, which agrees with previous proposals.[1] In contrast R 5020 but not progesterone blocked the action of RU 486 on both delivery and gap junctions, which supports the conclusions that the antihormone's effects are mediated through the progesterone receptor. The reason that progesterone is ineffectual but R 5020 blocks the action of RU 486 effects may be explained because of the higher affinity of the R 5020 compound for the progesterone receptor.[19]

In summary, our study suggests that progesterone may normally suppress myometrial activity and thus maintain pregnancy in rats by inhibiting a genome that controls the synthesis of gap junction proteins. Whether progesterone has a similar influence on the human myometrium remains to be resolved. However, the antiprogesterone RU 486 is also highly effective in interrupting pregnancy in women even in advanced stages,[20] and gap junctions are also present in large numbers in the human myometrium during term and preterm delivery.[4] Although these results do not preclude different control systems, they may indicate that similar mechanisms exist in humans even though the site of synthesis of progesterone during pregnancy (placenta versus ovary) is different in humans and rodents. The use of RU 486 has helped demonstrate that progesterone is required for the maintenance of both early and late stages of pregnancy in women.

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Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration

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The pharmacokinetic characteristics of the antiemetic drug metoclopramide and the antihistamine diphenhydramine have been determined in a chronically catheterized pregnant sheep preparation. Metoclopramide and diphenhydramine were administered by separate maternal and fetal intravenous infusions to a steady state as well as by maternal intravenous bolus dosing. Drug concentrations in the maternal and fetal plasma and the amniotic and tracheal fluids were measured by means of capillary gas-liquid chromatographic assay techniques. Both metoclopramide and diphenhydramine were excreted into tracheal fluid in substantial quantities. Tracheal metoclopramide concentrations were found to exceed fetal plasma levels by about fifteenfold while diphenhydramine attained maximal excretion in tracheal fluid of about five times that seen in fetal plasma. Drug levels were observed to accumulate slowly in amniotic fluid and eventually to exceed tracheal concentrations. The markedly elevated concentrations of these drugs in fetal lung fluid suggests that the fetal lung may be an important route of drug distribution, elimination, and excretion. (AM J OBSTET GYNECOL 1987;157:1286-91.)

Key words: Metoclopramide, diphenhydramine, placental transfer, tracheal fluid, amniotic fluid

There are numerous reports of the pharmacokinetic behavior of drugs in the fetus, conducted either in man or in animal species.[1] Most studies have concentrated on measurement of drug concentrations in the maternal and fetal vascular compartments, with fewer investigations also examining drug levels in fetal urine and/or the amniotic fluid. One potential route of drug excretion from the fetus that does not appear to have been studied is via fluid secreted from the fetal lung. Such fluid production occurs at a rate of approximately 4 ml/kg · hr in the fetal lamb,[2] with the fluid being either secreted into the amniotic cavity or swallowed.[2] The fluid appears to be formed primarily as a consequence of active chloride transport from plasma across the pulmonary epithelium.[3] In some respects the fluid resembles an ultrafiltrate of plasma, particularly in regard to low protein concentrations. However, the plasma/lung concentration ratios for several electrolytes differ substantially from those expected in a true ultrafiltrate.

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Metoclopramide is a potent antiemetic and gastric motility stimulator, structurally related to procainamide. It has been used before general anesthesia in labor to reduce the risk of maternal pulmonary aspiration of the stomach contents.[4] Diphenhydramine is an antihistaminic agent used in the treatment of allergies, nausea, and vomiting. It is also present in many nonprescription cough and cold remedies. Recent surveys indicate that about 10% to 20% of pregnant women take antihistamine-containing preparations at some point during pregnancy.[5] There is, however, limited information on the placental transfer of these drugs.[4, 6, 7]

In the course of pharmacokinetic studies of drug placental transfer in the chronically catheterized pregnant sheep we have collected and determined metoclopramide and diphenhydramine concentrations in maternal and fetal plasma and in amniotic and fetal lung fluids. In this report we describe a novel finding of marked accumulation of these drugs in fetal tracheal fluid in pregnant sheep.

Material and methods

Animal preparation. Studies were carried out on 11 pregnant sheep at 120 to 132 days' gestation. Surgery was performed with the animals under halothane anesthesia and silicone rubber catheters were implanted in a maternal femoral artery and vein and a fetal femoral artery, a lateral tarsal vein, the trachea,

Table I. Details of types and numbers of metoclopramide and diphenhydramine experiments

Experiment	*n*	*Ewe Nos.*
Maternal metoclopramide bolus injection of 40 mg	4	121, 127, 138, 140
Maternal metoclopramide infusion of 15 mg loading dose, then 0.21 mg/min for 90 min	4	60, 62, 98, 139
Fetal metoclopramide infusion of 5 mg loading dose, then 0.07 mg/min for 90 min	4	60, 62, 98, 139
Maternal diphenhydramine bolus injection of 100 mg	3	62, 98, 104
Maternal diphenhydramine infusion of 20 mg loading dose, then 0.67 mg/min for 90 min	4	121, 125, 133, 138
Fetal diphenhydramine infusion of 5 mg loading dose, then 0.17 mg/min for 90 min	4	121, 125, 133, 138

Table II. Mean ± SEM values for the ratio of drug concentration in tracheal fluid to that in fetal arterial plasma (TR/FA) and half-life values for drug in maternal and fetal arterial plasma and in tracheal fluid

Experiment	*TR/FA ratio*	*Maternal half-life (min)*	*Fetal half-life (min)*	*Tracheal fluid half-life (min)*
Maternal metoclopramide bolus	15.1 ± 0.7	87.5 ± 8.2	114.3 ± 15.7	127.9 ± 16.3
Maternal metoclopramide infusion	12.3 ± 1.7	55.6 ± 2.8	116.1 ± 17.1	94.5 ± 13.4
Fetal metoclopramide infusion	15.7 ± 1.7	83.5 ± 8.7	85.1 ± 20.7	81.8 ± 12.1
Maternal diphenhydramine bolus	2.8 ± 0.5	48.7 ± 3.6	47.3 ± 6.8	41.3 ± 1.8
Maternal diphenhydramine infusion	2.1 ± 0.4	51.3 ± 5.0	49.3 ± 11.0	38.5 ± 5.5
Fetal dephenhydramine infusion	4.2 ± 2.3	35.8 ± 5.5	53.8 ± 4.7	47.3 ± 5.3

and the amniotic cavity. The tracheal catheter (2.2 mm outside diameter) was inserted between adjacent tracheal rings, 1 cm below the larynx, with the catheter tip lying just above the carina. It did not impair the free flow of fluid out of the lung via the upper airway. After surgery, the ewes were placed in pens in the company of other sheep. On an experimental day, the sheep was transferred to a monitoring cart adjacent to the holding pen.

Experimental protocol. A total of 23 experiments were conducted on the 11 sheep. Details of the experiments are given in Table I. Experiments were performed from 3 to 14 days (mean = 7.2 ± 1.0) after surgery. The two drugs were administered intravenously to the ewe or fetus. In the case of infusions of metoclopramide and diphenhydramine, the paired maternal and fetal infusions to each animal were given 48 hours apart. In the other situations involving multiple experiments in the same animal, a minimum of 48 hours elapsed between separate experiments.

Infusions of drug were carried out with a Harvard infusion pump (Model 944, Harvard Apparatus, Millis, Massachusetts); the volume infusion rate was 0.017 ml/min. Metoclopramide hydrochloride (Reglan Injectable, 5 mg/ml, A. H. Robins Co., Montreal, Quebec, Canada) and diphenhydramine hydrochloride (Benadryl Injectable, 50 mg/ml, Parke-Davis, Scarborough, Ontario, Canada) were diluted in 0.9% saline solution to the appropriate concentration immediately before administration. For the infusion studies, samples were simultaneously withdrawn at −5, 5, 15, 30, 45, 60, 75, 90, 95, 100, 105, 110, 120, 135, 150, 180, 210, 240, 270, 300, 330, and 390 minutes from the maternal (2.5 ml) and fetal (1.5 ml) femoral arterial, amniotic (1.5 ml), and tracheal (1.5 ml) catheters. For bolus metoclopramide injections, the same catheters were sampled at −5, 1, 3, 6, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, and 420 minutes. For the diphenhydramine bolus injections, only 5- and 10-minute samples were collected in the first 10 minutes; otherwise the sampling regimen was identical.

In each experiment, the volume of fetal blood collected was replaced by an equal volume of maternal blood at intervals during the study. Plasma, tracheal and amniotic samples were stored at −20° C until analysis.

Physiologic measurements. During the experimental period, fetal arterial, tracheal, and amniotic pressures were measured with strain-gauge manometers (Statham Model P23Db, Gould Inc., Oxnard, California). Fetal heart rate was measured from the arterial pulse pressure by means of a cardiotachometer (Model 9857, Sensormedics, Anaheim, California). Fetal P_{O_2}, P_{CO_2}, and pH were measured with Il Micro 13 blood gas analyzer (Instrumentation Laboratories, Lexington, Massachusetts). The fetal effects of metoclopramide have previously been reported in preliminary form.[7] The fetal cardiorespiratory and behavioral effects of diphenhydramine are currently under investigation.

Drug analysis. Drug measurement of metoclopramide and diphenhydramine was accomplished with the use of capillary gas-liquid chromatographic methods, in which electron-capture and nitrogen/phosphorus selective detection, respectively, were used.[6, 8]

Pharmacokinetic analysis. The experiments re-

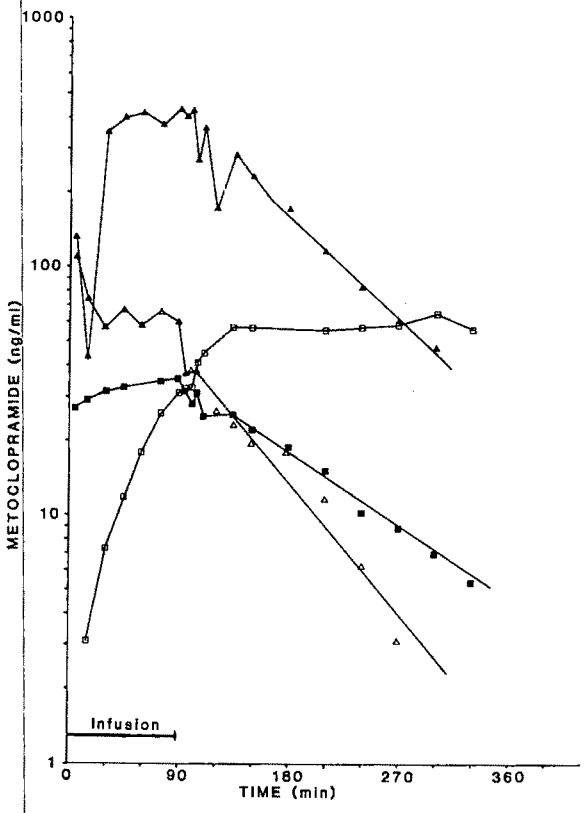


Fig. 1. Representative semilogarithmic plots of the maternal (△——△) and fetal (■——■) plasma, tracheal (▲——▲), and amniotic (□——□) metoclopramide concentrations versus time profiles in a pregnant ewe and fetus after a 15 mg intravenous bolus and an infusion of 0.21 mg/min for 90 minutes to the ewe.

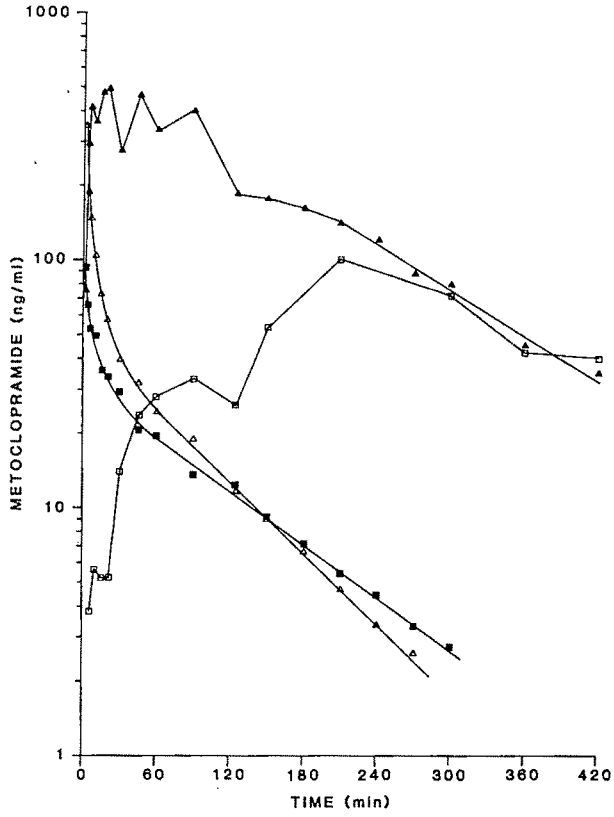


Fig. 2. Semilogarithmic plots of maternal (△——△) and fetal (■——■) plasma, tracheal (▲——▲), and amniotic (□——□) metoclopramide concentrations versus time profiles after a 40 mg maternal intravenous bolus.

ported in this paper were part of larger studies on the overall pharmacokinetics of metoclopramide and diphenhydramine in pregnant sheep. The pharmacokinetic parameters estimated from the plasma drug concentration versus time data from the bolus injection studies of the two drugs are presented in other reports.[7,8] In the present study, we calculated the maternal and fetal half-life for the drugs after bolus injection by linear regression analysis of the drug concentration versus time profile during the terminal elimination phase. The maternal volume of distribution (V_d) and elimination rate constant (β) data from these studies were also used to calculate the drug infusion rates (k_o) used in the steady-state infusion experiments by use of the equation:

$$C_{ss} = k_o/(V_d \times \beta)$$

where C_{ss} equals the desired maternal steady-state plasma concentration of the drug. The data from the infusion studies are being analyzed with use of a two-compartment open model of drug clearance in pregnancy proposed by Szeto[1] and will be the subject of further reports when the studies are completed. For the present article, we calculated maternal and fetal half-lives for the drugs in the infusion studies from linear regression analysis of the terminal elimination phase of the drug concentration versus time data after cessation of the infusion.

Results

Figs. 1 and 2 illustrate representative semilogarithmic concentration versus time profiles obtained for metoclopramide in each of the four fluid samples in experiments involving maternal infusion to a steady state (Fig. 1) and maternal intravenous bolus injection (Fig. 2). In both cases, the drug concentration in tracheal fluid was approximately tenfold to fifteenfold higher than in fetal plasma and a similar situation occurred with infusion of metoclopramide directly to the fetus (Table II). With the maternal metoclopramide infusions the levels in tracheal fluid reached their peak within 45 minutes of dosing, whereas with fetal infusion this occurred within 15 minutes. After cessation of the infusion, metoclopramide levels in tracheal fluid followed an exponential decline but with large periodic fluctuations for the first 1 to 2 hours (Figs. 1 and 2). However, throughout the entire time course of the experiment, the metoclopramide concentration in tra-

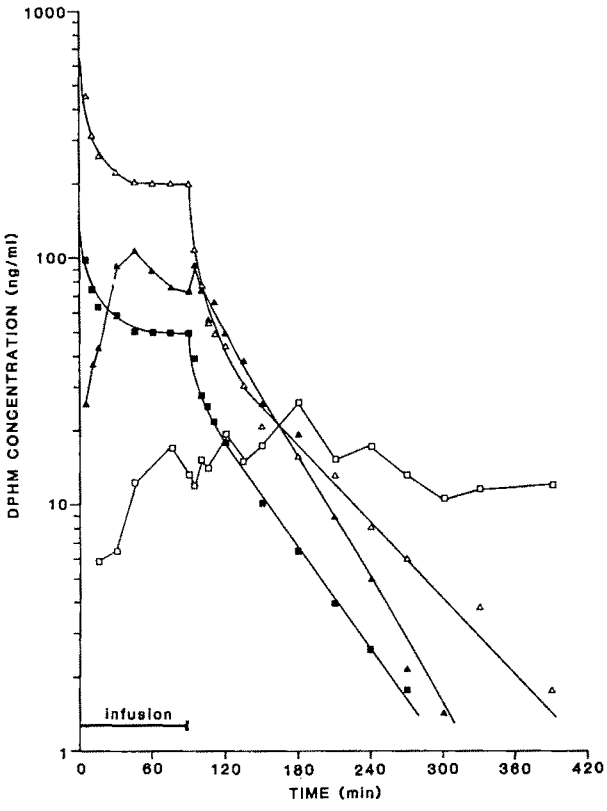


Fig. 3. Representative semilogarithmic plots of the maternal (△——△) and fetal (■——■) plasma, tracheal (▲——▲) and amniotic (□——□) diphenhydramine *(DPHM)* concentrations versus time profiles in a pregnant ewe and fetus after a 20 mg intravenous bolus and an infusion of 0.67 mg/min for 90 minutes to the ewe.

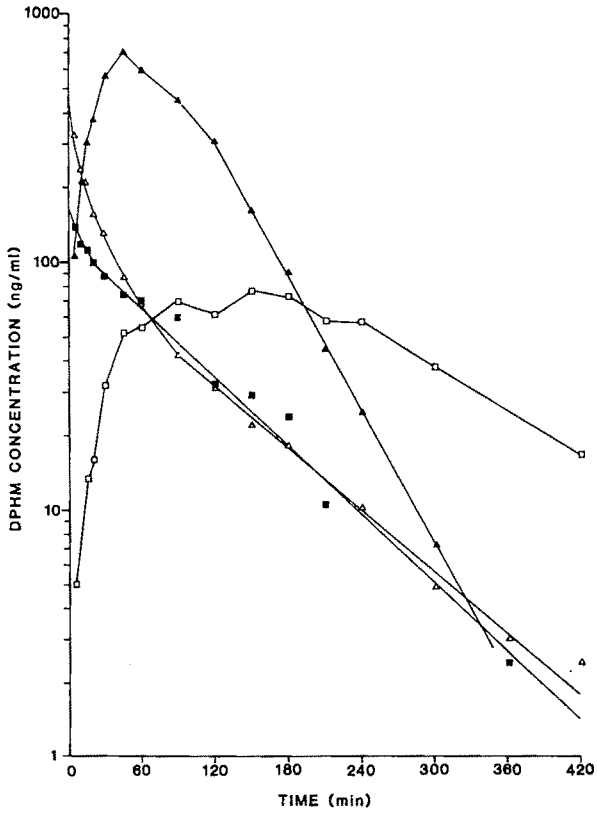


Fig. 4. Semilogarithmic plots of maternal (△——△) and fetal (■——■) plasma, tracheal (▲——▲) and amniotic (□——□) diphenhydramine *(DPHM)* concentrations versus time profiles after a 100 mg maternal intravenous bolus.

cheal fluid remained at least tenfold to fifteenfold higher than either the fetal or maternal plasma concentrations. On the other hand, the metoclopramide concentration in amniotic fluid rose progressively during the infusion period, reaching a plateau by 30 minutes after termination of the infusion (Fig. 1) and 210 minutes after bolus injection (Fig. 2). By 210 to 270 minutes, metoclopramide levels in the tracheal and amniotic fluid were similar. Also the highest concentration ultimately achieved in amniotic fluid was similar to the highest fetal plasma metoclopramide concentration observed in the initial phases of the experiments (mean ratio = 1.3 ± 0.2).

Figs. 3 and 4 show representative semilogarithmic plots of the concentration versus time profiles for diphenhydramine in each of the four fluid samples examined after maternal intravenous infusion to a steady state (Fig. 3) and maternal intravenous bolus injection of the drug (Fig. 4). As with metoclopramide, the levels of diphenhydramine in tracheal fluid were consistently higher than in fetal plasma. However, the extent of accumulation was less for diphenhydramine, with the tracheal fluid to fetal plasma ratio averaging 2 to 4 (Table II). A maximum ratio of ~5 was observed in the experiment illustrated in Fig. 4. The decline of the tracheal diphenhydramine concentration followed an exponential process; however, the marked fluctuations associated with metoclopramide excretion in tracheal fluid were not observed. Diphenhydramine in amniotic fluid reached plateau levels 45 to 90 minutes after drug administration and the amniotic diphenhydramine concentration exceeded those in fetal and maternal plasma and tracheal fluids by 70 to 200 minutes. However, the maximum level observed in amniotic fluid was substantially less than the highest diphenhydramine concentration in fetal plasma in each experiment (mean ratio = 0.3 ± 0.1).

The elimination of both drugs from maternal and fetal plasma was rapid (Table II). For metoclopramide, the average apparent terminal half-life in the fetus exceeded that in the ewe for all three types of experiments, and the overall average fetal value of 105.2 ± 10.3 minutes was significantly ($p < 0.05$, paired t test) higher than the mean maternal value of 75.5 ± 5.7 min. The apparent half-life of metoclopramide in tracheal fluid was very similar to that in fetal

plasma. For diphenhydramine, there were no consistent differences in half-life values in maternal and fetal plasma and tracheal fluid. After a bolus injection of metoclopramide and diphenhydramine, drug concentrations in amniotic fluid were declining by the end of the sampling period (Figs. 2 and 4). In contrast, with infusion to a steady state, metoclopramide concentrations in amniotic fluid were still at a plateau level at the end of the experiment (Fig. 1). This was sometimes the case for diphenhydramine (Fig. 3), but in most instances the amniotic fluid concentration of the drug was declining by the end of the experiment.

Comment

In adults, metabolism, excretion, and irreversible binding of many drugs, particularly amine compounds, by the lung are important processes in total body clearance of these agents, in part because the lung receives all of the cardiac output.[9] In the fetus, the percentage of combined ventricular output distributed to the lung is low (~6%), as is pulmonary blood flow (~28 ml/min · kg).[10] However, in spite of this, the results of the present study demonstrate marked accumulation of two amine drugs, metoclopramide and diphenhydramine, in fetal lung fluid. The overall average tracheal/fetal plasma concentration ratios for metoclopramide and diphenhydramine were 14.4 ± 1.0 and 3.0 ± 0.6, respectively. We do not yet have any data to explain this difference in the ratio, but the higher plasma protein binding in human adults reported for diphenhydramine (~80%),[11] as compared with metoclopramide (~20%),[12] may be involved. The cell types in the adult lung that are responsible for accumulation and metabolism have not been identified for all drugs, but pulmonary endothelial cells are known to be important in the uptake of a number of basic amine drugs.[13] We do not yet have any data to suggest specific mechanisms in the fetus. Also, we are not aware of any reports of accumulation of other drugs in fetal tracheal fluid. However, we have preliminary data indicating that the tracheal fluid/fetal arterial plasma concentration ratios for estradiol and progesterone are 0.40 and 0.22, respectively, suggesting no significant accumulation of these steroids in fetal tracheal fluid (Rurak DW, Leung PK, unpublished observations).

Even with the low rate of pulmonary blood flow in the fetus, the delivery of these drugs to the lung via the pulmonary circulation would appear to be more than sufficient to account for the levels observed in tracheal fluid. We base this argument on rough calculations done for an experiment involving maternal infusion of metoclopramide to a steady state. We have assumed a fetal weight of 2 kg, a lung fluid volume of 30 ml/kg,[2] a tracheal fluid excretion rate of 4 ml/hr · kg,[2] and a lung blood flow of 56 ml/min.[10] With these values and the measured metoclopramide concentrations in fetal plasma and tracheal fluid, it can be calculated that during the 90-minute infusion period approximately 13% of the metoclopramide delivered to the lungs would have to be taken up to account for the accumulation of the drug in tracheal fluid. However, it also appears that the rate of pulmonary uptake of metoclopramide would not be constant over the infusion period. Rather it would be greatest during the first ~5 minutes after the infusion is started, when extraction would have to be ~74%, and would thereafter decline progressively as the metoclopramide levels in tracheal fluid reached a plateau level. After cessation of the infusion, it would appear that further uptake of the drug by the lung would be very low. In essence, whatever the mechanism involved in metoclopramide accumulation in tracheal fluid, it appears to operate to create approximately a fifteenfold gradient in drug concentration between this fluid and fetal plasma. A large proportion of the total amount of metoclopramide taken up by the lung would be in the initial phase of drug administration to create the gradient. Once this is achieved, further uptake of metoclopramide is required only to replace that lost via tracheal fluid excretion from the airway. Of course, the above arguments apply equally well to diphenhydramine, with the difference being that the pulmonary extraction of this drug would have to be much less (~2% over the 90-minute infusion period) than for metoclopramide, given its much lower levels in tracheal fluid.

The accumulation of metoclopramide and diphenhydramine in fetal lung fluid could have several potential consequences. First, tracheal fluid could be a significant route of drug elimination from the fetus. For example, with metoclopramide, during the first 120 minutes after the start of the maternal infusion, approximately 3.2 μg of intact drug was excreted in tracheal fluid, assuming a rate of tracheal fluid production of 4 ml/hr · kg. This compares with a calculated total renal excretion for metoclopramide of 3.6 μg, assuming a glomerular filtration rate of 1 ml/min · kg[14] and no significant renal tubular resorption of metoclopramide. Although in vitro studies of the fetal liver have demonstrated the relatively early ontogenic appearance of oxidative, hydrolytic, and conjugative enzyme systems in man and other primates,[15] in vivo data from fetal lambs indicate a low hepatic extraction of propranolol.[16] Hence it may be that excretion of intact drug via lung fluid and urine is more important in overall fetal drug elimination. Second, since fetal lung fluid either enters the amniotic fluid compartment or is swallowed, a high drug concentration in tracheal fluid could contribute significantly to amniotic fluid levels. This could be particularly important in the case of metoclopramide (Figs. 1 and 2). Alternatively, fetal inges-

tion of tracheal fluid could be an important route for recycling of drug within the fetus. In this regard, the larger half-life of metoclopramide in fetal plasma compared with that in the ewe could be due, at least in part, to reuptake of the drug from the fetal lung into plasma. Finally, it would appear that at least some of the cellular elements within the lung could be exposed to very high drug concentrations. This would not be predicted by estimates of relative fetal drug exposure based on fetal/maternal plasma concentration ratios.[16] In summary, this potential route of drug excretion in the fetus deserves further investigation.

We thank A. H. Robins Canada Inc. (Montreal, Quebec, Canada) for the supply of metoclopramide.

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Corrections

In the article by Edelstone et al., entitled "Effects of maternal anemia on cardiac output, systemic oxygen consumption, and regional blood flow in pregnant sheep" (AM J OBSTET GYNECOL 1987;156:740-8), the fourth sentence of the first paragraph of the Methods section should have been: "Local anesthesia (Xylocaine, 2%) was used for these catheter insertions."

In the article by Hustin and Schaaps, entitled "*Echocardiographic* and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy" (AM J OBSTET GYNECOL 1987;157:162-8), the title should have been "*Echographic* and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy."

Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus

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Atrial natriuretic peptide is a potent diuretic and vasorelaxant peptide secreted from the cardiac atria of adult mammals in response to increased intravascular volume. In sheep, fetal plasma levels of atrial natriuretic peptide are significantly greater than maternal levels, but the factors that control fetal release of atrial natriuretic peptide have not been defined. The present studies were conducted to determine whether the fetus responds to increased intravascular volume by atrial natriuretic peptide secretion. The mean (± standard error of the mean) atrial natriuretic peptide of basal fetal plasma (115 ± 24 pg/ml) in chronically catheterized ovine fetuses (131 ± 1 days' gestation) was significantly greater than atrial natriuretic peptide levels of basal maternal plasma (56 ± 12 pg/ml). In response to successive 30-minute intravenous infusions of 0.9% saline solution at 0.5 and 1.0 ml/kg/min, fetal plasma atrial natriuretic peptide significantly increased to a maximum of 409 ± 72 pg/ml during the high-dose saline infusion and returned to 234 ± 83 pg/ml during a 30-minute recovery period. The increase in fetal plasma atrial natriuretic peptide was significantly correlated with the volume of saline solution infused ($r = 0.68$). During the saline infusion, a significant increase in fetal blood volume and heart rate was noted. Fetal blood pressure, maternal plasma levels of atrial natriuretic peptide, and fetal and maternal plasma osmolality remained unchanged. Despite elevated basal levels of plasma atrial natriuretic peptide, the third-trimester ovine fetus responds to increases in intravascular volume with a further increase in atrial natriuretic peptide secretion. (Am J Obstet Gynecol 1987;157:1292-7.)

Key words: Blood volume, atrial natriuretic peptide, cardiac

Atrial natriuretic peptide comprises a group of biologically active peptides synthesized by mammalian atrial cardiocytes. Histochemical studies suggest that specific atrial granules store and release the polypeptide, which is consistent with a secretory function for the atrial cardiocyte. Atrial natriuretic peptide has been demonstrated to produce a significant natriuresis and diuresis in mammals, principally via an increase in glomerular filtration; a tubular effect of atrial natriuretic peptide is controversial. Atrial natriuretic peptide also has significant effects on systemic blood pressure and renin, aldosterone, and arginine vasopressin secretion. In adult mammals, increased secretion of atrial natriuretic peptide occurs in response to intravascular volume expansion, with atrial stretch believed to be the actual stimulus.[1,2]

Although the secretion, function, and metabolism of atrial natriuretic peptide have been studied in a variety of species of adult mammals, little is known of the secretion of atrial natriuretic peptide in the fetal mammal. The present studies sought to determine if the chronically catheterized ovine fetus responds to an intravascular volume load with increased secretion of atrial natriuretic peptide.

Methods

Animals and surgery. Five studies were performed in four mixed-breed Western cross-bred ewes with singleton fetuses ranging in age from 127 to 134 days (mean gestational age, 131 ± 1 day). Animals were housed indoors in individual steel study cages and maintained under a controlled lighting regimen of 12 hours of light and 12 hours of darkness. Both food (alfalfa pellets) and water were available ad libitum, except for the withholding of food during the 24-hour period immediately before surgery.

Anesthesia for surgery was induced by an intramuscular injection of ketamine hydrochloride (20 mg/kg) plus atropine sulfate (30 μg/kg) and subsequently maintained by a continuous intravenous infusion of ketamine (15 mg/hr · kg^{-1}). Lidocaine (Xylocaine) (1%) was

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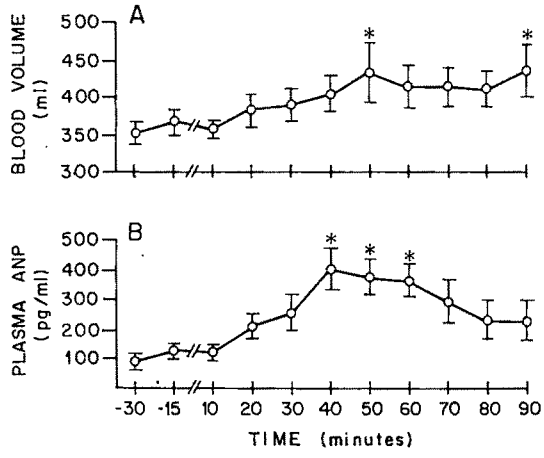


Fig. 1. Mean ± SEM fetal blood volume (**A**) as determined by chromium 51 dilution and plasma atrial natriuretic peptide (**B**) during basal (−30 to 0 minutes), low- and high-dose saline infusions (0 to 30 and 30 to 60 minutes, respectively), and recovery periods (60 to 90 minutes). (*$p < 0.05$ versus mean basal values; *ANP* = atrial natriuretic peptide.)

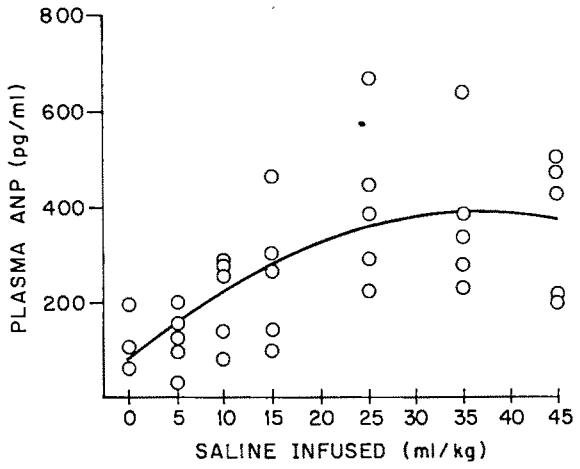


Fig. 2. Polynomial relation of plasma atrial natriuretic peptide concentration to volume of saline solution infused. (*ANP* = Atrial natriuretic peptide.)

Table I. Maternal arterial values before and at conclusion of saline infusions to the fetus

	Basal	*Infusion*
pH	7.48 ± 0.02	7.50 ± 0.01
P_{O_2} (mm Hg)	95 ± 4	98 ± 4
P_{CO_2} (mm Hg)	33 ± 2	31 ± 1
Hematocrit (%)	29.4 ± 1.3	29.0 ± 1.5
Osmolality (mosm/kg H_2O)	301 ± 2	301 ± 2
Atrial natriuretic peptide (pg/ml)	55.6 ± 11.6	43.1 ± 4.7

applied locally before all incisions. The uterus was exposed by a midline abdominal incision, and a small hysterotomy was performed to provide access to a single fetal hindlimb. Tygon catheters (1 mm inner diameter; 1.8 mm outer diameter) were placed in the fetal dorsal hindlimb vein and artery and threaded to the inferior vena cava and abdominal aorta, respectively. Before hysterotomy closure, a plastic catheter (Corometrics Medical Systems, Wallingford, Conn.) was sutured to the distal tip of the fetal hindlimb to provide access to the amniotic cavity. The uterus, maternal fascia, and skin were closed in separate layers with sutures. The maternal femoral artery and vein in one leg were catheterized, and the catheters were threaded to the aorta and inferior vena cava, respectively. All catheters were exteriorized to the maternal flank and placed in a cloth pouch sewn to the ewe's flank.

Fetal vascular catheters were maintained patent by filling the catheter deadspace (1 ml) with 1000 U/ml sodium heparin and sealing with sterile plastic caps. Maternal vascular catheters were filled with heparinized saline solution (10 μU/ml), and both fetal and maternal catheters were flushed daily. Immediately after surgery and twice daily for the succeeding 5 days, fetuses received an intravenous infusion of oxacillin (30 mg/kg) and gentamicin (4 mg/kg). Oxacillin (400 mg) and chloramphenicol (1 gm) were also administered into the maternal vein and amniotic cavity.

Experimental protocol. All experiments were performed on unanesthetized ewes standing in the same individual cages in which they were maintained. Fetal arterial blood pH values were assessed, and studies were undertaken only if the fetal arterial pH was greater than 7.3. Before an initial 60-minute basal period, 2 ml of fetal blood was withdrawn, labeled with chromium 51 (ICN Radiochemicals, Irvine, Calif.), and reinjected into the fetal vein as described by Brace.[3] After equilibration for 45 minutes, fetal and maternal blood samples were collected at 30 and 15 minutes before initiation of saline infusion. At time 0, a fetal intravenous infusion of 0.9% saline solution (0.5 ml/kg/min) was initiated and maintained for 30 minutes. At time +30, the saline infusion was increased to (1.0 ml/kg/min) for an additional 30 minutes. At +60 minutes, the saline infusion was discontinued, and the fetus was observed for a 30-minute recovery period. Infusion rates were based on estimated fetal body weights from the formula: fetal body weight (kg) = (0.096 × gestational age [days]) − 9.2228, as described by Robillard and Weitzman.[4]

Fetal arterial blood samples (2.5 ml) were collected at 10-minute intervals after initiation of the saline infusion. Blood samples were placed into iced tubes containing Trasylol (250 kallikrein units/ml blood), trypsin inhibitor (40 μU/ml), and K_2 ethylenediaminetetraace-

Table II. Fetal arterial values during the control period, low- and high-dose saline infusions, and recovery period

	Control (min)		*Saline (0.5 ml/kg/min) (min)*		
	−30	*−15*	*10*	*20*	*30*
pH	7.38 ± 0.01	7.37 ± 0.01			7.38 ± 0.01
P_{O_2} (mm Hg)	22 ± 2	21 ± 2			20 ± 2
P_{CO_2} (mm Hg)	49 ± 1	48 ± 1			47 ± 1
Systolic (mm Hg)	56.7 ± 3.4	57.2 ± 3.1	57.1 ± 3.3	58.2 ± 4.9	58.7 ± 3.2
Diastolic (mm Hg)	35.1 ± 1.3	34.0 ± 1.0	34.3 ± 1.1	36.4 ± 0.7	37.5 ± 1.0
Mean (mm Hg)	43.1 ± 1.1	42.4 ± 1.4	43.5 ± 0.9	44.8 ± 1.8	44.9 ± 0.6
Heart rate (bpm)	172 ± 4	168 ± 5	181 ± 10	197 ± 20	194 ± 17
Hematocrit (%)	37.0 ± 1.8	36.7 ± 1.6	36.2 ± 1.8	35.8 ± 1.6*	35.7 ± 1.7*
Osmolality (mosm)	295 ± 2	296 ± 2			296 ± 2

*$p < 0.05$ versus mean basal value.

tic acid (1 mg/ml). Chromium 51 activity was determined in a 300 μl aliquot of each sample. Hematocrit determinations were performed in triplicate on each blood sample. All samples were centrifuged immediately. Osmolality was measured in aliquots of the prepared plasma; residual plasma was stored at −20° C until assayed for atrial natriuretic peptide. In addition, 0.5 ml of blood was collected into heparinized tuberculin syringes for immediate measurements of pH, P_{O_2}, and P_{CO_2}. The total volume of fetal blood withdrawn at each sample (3.0 ml) was replaced with an equal volume of heparinized maternal blood withdrawn before the study. Fetal arterial blood pressure and amniotic cavity pressures were monitored continuously by means of a Beckman R-612 physiologic recorder (Beckman, Palo Alto, Calif.) with Statham P23 transducers. Fetal blood pressure was corrected for amniotic fluid pressures, and fetal heart rate was computed from the arterial pulse. Repeat samples of maternal arterial blood were drawn at 20 and 30 minutes of the 1.0 ml/kg/min infusion.

Analytic methods. Plasma determinations of atrial natriuretic peptide were performed by a recently described[5] radioimmunoassay method developed in our laboratory that uses synthetic human atrial natriuretic peptide (1 to 28) obtained from Bachem, Inc. (Torrance, Calif.). Briefly, atrial natriuretic peptide was coupled with bovine thyroglobulin by means of carbodiimide, and the coupled material was used to generate antisera in New Zealand white rabbits. Radiolabeled atrial natriuretic peptide was prepared with Iodogen (Pierce Chemical Co, Rockford, Ill.). Plasma samples were acidified with 100 μl of 1N hydrochloric acid and extracted on C18 Sep-Pac columns (Waters Associates, Milford, Mass.). Columns were washed with 10 ml methanol followed by 10 ml triethylamine acetate buffer (pH 4.0). Acidified plasma samples (1 ml) were added slowly to Sep-Pac columns, and the columns were washed with 10 ml triethylamine acetate buffer (pH 4.0). The absorbed peptide was eluted with 4.0 ml of 80% methanol in triethylamine acetate buffer, and the eluates were dried in a Speed-Vac concentrator (Savant Instruments, Hicksville, New York). Dried fractions were stored frozen (−20° C) until dissolved in radioimmunoassay buffer immediately before assay.

The entire atrial natriuretic peptide-radioimmunoassay procedure was performed at 4° C. The assay buffer was 0.01 mol/L phosphate-buffered saline solution containing 0.1% bovine serum albumin and 0.1% triton X-100, with a pH of 7.4. Duplicate 50 or 100 μl plasma extracts or standards were mixed with 100 μl of antiserum in a total volume of 300 μl and incubated overnight at 4° C. Then 100 μl of iodinated tracer (~5000 cpm) was added, mixed, and incubated overnight at 4° C. Bound and free fractions were separated by adding goat antirabbit γ-globulin (50 μl of 1:10 dilution) and normal rabbit serum (50 μl of 1:50 dilution). Polyethylene glycol 6000 (75 μl of 25% solution) was added, and the tubes were centrifuged (2000 rpm at 4° C) for 30 minutes. The supernatant was aspirated, and the radioactivity in the precipitates was measured in a γ-counter.

A typical standard curve ranged from 1 to 500 pg/tube, which was plotted as B/Bo versus picograms per tube. The sensitivity of the assay, which was defined as the least amount of atrial natriuretic peptide distinguishable from 0 at the 95% confidence limit, was 2 pg/tube. Cross reactivities of our antiserum with a variety of atrial natriuretic peptide analogs were human atrial natriuretic peptide (one to 28 amino acids) 100%, rat atrial natriuretic peptide (one to 28 amino acids) 43%, rat atriopeptin I (five to 25 amino acids) 1%, rat atriopeptin II (five to 27 amino acids) 0.2%, rat atriopeptin III (five to 28 amino acids) 0.1%, and rat atriopeptide (13 to 28 amino acids) <0.1%.

The mean recoveries of synthetic human atrial natriuretic peptide added to pools of either ovine adult plasma (n = 10) or fetal plasma (n = 10) were similar

Saline (1.0 ml/kg/min) (min)			*Recovery (min)*		
40	*50*	*60*	*70*	*80*	*90*
		7.36 ± 0.01			7.37 ± 0.01
		21 ± 3			20 ± 2
		46 ± 1			45 ± 1*
59.8 ± 3.1	61.5 ± 3.4	60.0 ± 3.1	57.6 ± 2.9	56.4 ± 2.4	57.2 ± 2.8
36.0 ± 0.4	37.5 ± 1.4	36.4 ± 0.7	35.2 ± 1.4	34.0 ± 1.3	34.4 ± 1.3
45.2 ± 0.3	45.5 ± 1.4	44.6 ± 1.7	43.4 ± 0.9	42.4 ± 1.6	42.4 ± 1.2
191 ± 12	203 ± 15	190 ± 14	212 ± 17*	196 ± 13	204 ± 15*
35.1 ± 1.4*	35.1 ± 1.3*	35.3 ± 1.5*	35.7 ± 1.4*	35.7 ± 1.5*	35.2 ± 1.5*
		295 ± 2			297 ± 2

(81% ± 6% and 85% ± 5%, respectively). Recovery of iodine 125–labeled atrial natriuretic peptide also was similar (78% ± 3%). The intra-assay and interassay coefficients of variation were 11% and 13%, respectively. All values of ovine atrial natriuretic peptide concentrations represent immunoreactive human atrial natriuretic peptide.

Blood pH, P_{O_2}, and P_{CO_2} values were determined at 39° C with a Radiometer BM 33 MK2-PHM 72 MK2 acid-base analyzer system (Radiometer Co., Copenhagen, Denmark). Plasma and urine osmolalities were measured by freezing-point depression on an Advanced-Digimatic osmometer (Model MO, Advanced Instruments, Inc., Needham Heights, Mass.). Chromium 51 activity was determined on a 1282 Compugamma γ-counter (LKB Wallac, Turku, Finland). Blood volume was determined as described by Brace[3]; corrections were made for the removal of chromium 51 activity caused by fetal blood sampling.

Statistics. All values are expressed as the mean ± standard error of the mean. Differences over time were assessed by one-way analysis of variance for repeated measures. Whenever a significant F test of $p < 0.05$ was observed, comparisons of the control means to each of the other group means were performed by the Dunnett test ($p < 0.05$). Maternal basal and infusion plasma levels were compared by paired *t* test.

Results

Maternal. Maternal arterial blood pH, P_{O_2}, P_{CO_2}, and hematocrit values, as well as plasma osmolality and atrial natriuretic peptide concentrations, are summarized in Table I. None of the maternal parameters changed significantly during the saline infusion to the fetus.

Fetal. There was no change from mean basal values of fetal arterial pH (7.37 ± 0.09), P_{O_2} (22 ± 2 mm Hg), systolic (57 ± 3 mm Hg), diastolic (35 ± 1 mm Hg), or mean (43 ± 1 mm Hg) blood pressure, or plasma osmolality (296 ± 2 mosm) during the saline infusions to the fetus (Table II). There was a small but significant decrease in fetal P_{CO_2} during the recovery period. Fetal heart rate tended to increase during the saline infusion, although a statistically significant increase was not noted until the recovery period. Mean fetal hematocrit level decreased significantly from a basal value of 37.0% ± 1.8% to 35.1% ± 1.3% during the saline infusion period (Table II).

As shown in Fig. 1, *A*, fetal blood volume increased significantly from a basal value of 359 ± 16 to a peak value of 434 ± 46 ml during the high-dose saline infusion. Basal levels of fetal plasma atrial natriuretic peptide (115 ± 24 pg/ml) were significantly greater than were basal maternal levels. As shown in Fig. 1, *B*, fetal plasma atrial natriuretic peptide significantly increased to a maximum of 409 ± 72 pg/ml during the saline infusion and returned to basal levels during the recovery period. The increase in fetal plasma atrial natriuretic peptide was significantly correlated with the volume of saline infused by a second-order polynomial relation ($r = 0.68$; $p < 0.001$; Fig. 2):

$$y = -5.83\,x^2 + 85.12\,x + 81.65$$

Comment

In the present study, basal levels of plasma atrial natriuretic peptide were significantly higher in the fetus than in the maternal ewe. This could be explained by a relative increase in the rate of fetal atrial natriuretic peptide secretion or a decrease in the rate of fetal metabolism of atrial natriuretic peptide when compared with the ewe. We[6] previously demonstrated an extremely rapid plasma clearance of atrial natriuretic peptide in the ovine fetus (94 ± 7 ml/min/kg), which suggests that the metabolic clearance of atrial natriuretic peptide is not reduced in the fetus. Direct information regarding the fetal production of atrial natriuretic peptide is not yet available. Fetal cardiac output and right heart function are proportionately

increased when compared with the adult, potentially stimulating atrial natriuretic peptide secretion via an increase in fetal atrial distention. In rats[7] a regulatory and permissive role for glucocorticoids and mineralocorticoids in the release of atrial natriuretic peptide has also been demonstrated. The unique fetal steroid endocrine milieu associated with increased plasma concentrations of deoxycorticosterone, progesterone, and aldosterone may potentiate fetal atrial natriuretic peptide secretion.

Despite basal fetal plasma levels of atrial natriuretic peptide twofold greater than maternal levels, the fetus responded to saline infusion with a further increment in plasma concentration. Thus despite what is likely a basal hypersecretion of atrial natriuretic peptide, the fetus retains significant atrial natriuretic peptide secretory reserves. The plasma atrial natriuretic peptide response to volume loading is consistent with the response to volume loading reported in adult mammals.[9] Whether the increase in plasma atrial natriuretic peptide has significant renal effects in the fetus is unknown at present. However, in a study of the effects of intravascular saline infusion (50 ml over 30 minutes) to the ovine fetus, Hurley et al.[8] demonstrated a significant increase in the fetal glomerular filtration rate and a decrease in fractional sodium resorption. These findings are consistent with reported renal actions of atrial natriuretic peptide, including a diuresis and natriuresis associated with increased glomerular filtration.

In the present experiments, a significant decrease occurred in the fetal hematocrit level in response to the successive intravenous saline infusions (Table II). This was not caused by fetal blood sampling, since each fetal sample was replaced euvolemically with maternal blood. Rather, the decrease in hematocrit level resulted from fetal plasma volume expansion after intravascular saline solution. Consequently, fetal blood volume, as calculated by chromium 51–labeled red cell dilution, increased progressively during the infusion. Presumably, the blood volume expansion and subsequent atrial stretch stimulate an increase in atrial natriuretic peptide secretion.

A significant increase in fetal heart rate was noted during the saline infusions to the fetus. Fetal systolic, diastolic, and mean blood pressure did not change significantly during the infusions despite the increase in plasma concentration of atrial natriuretic peptide. Although a hypotensive response to atrial natriuretic peptide infusion has been noted in in vitro preparations, this has not been a consistent observation in vivo.[9] A marked hypotensive action of atrial natriuretic peptide has been demonstrated in models of increased renin/angiotensin, apparently as a result of atrial natriuretic peptide antagonism of angiotensin-induced vasoconstriction.[9] Although the ovine fetus demonstrates increased plasma renin activity,[10] plasma angiotensin II levels are comparable with values in adult ewes.[11] The role of basal levels of angiotensin in blood pressure homeostasis and thus the susceptibility to vasodilating effects of atrial natriuretic peptide appear to be minimal in the ovine fetus.[12]

In the conscious adult sheep preparation, hypotensive effects of atrial natriuretic peptide apparently result from a decrease in cardiac output.[13] Relaxation of venous smooth muscle with a reduction in venous return and stroke volume results in a reflex increase in peripheral resistance and an increase in heart rate. The increase in heart rate noted in the present study is consistent with these actions. A hypotensive effect might have been mitigated by fetal reflex arterial constriction and the saline-induced blood volume expansion.

Fetal basal plasma osmolality and intravascular water are dependent on maternal levels; changes in maternal hydration induce transplacental water flows that acutely alter fetal homeostasis. We previously demonstrated that in response to maternal plasma hyperosmolality and the resultant fetal blood volume contraction, there is an increase in fetal arginine vasopressin secretion that decreases fetal urine and lung fluid flows.[14, 15] The fetus preserves body water at the expense of amniotic fluid production.[16] It is probable that a counterregulatory endocrine system, such as atrial natriuretic peptide, exists to adjust to acute fetal blood volume expansion. Whether fetal atrial natriuretic peptide modulates in utero urine production and lung fluid secretion remains to be determined.

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Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys

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Insulin concentrations, in response to an intravenous glucose bolus after a 24-hour fast, have been studied in female rhesus monkeys in which the circulating levels of androstenedione and testosterone or estrone and estradiol have been increased for as long as 4½ years. No significant differences were observed in the basal insulin or C-peptide concentrations in the androgen or estrogen-treated animals compared with each other or with normal cycling, nontreated control animals. The insulin and C-peptide responses to intravenous glucose were similar in control and androgen-treated monkeys. Compared with both the control and androgen-treated monkeys, the responses of the estrogen-treated monkeys tended to be lower but were not significantly different. The glucose disappearance rate after the intravenous glucose bolus was not significantly different in androgen and control monkeys but was significantly slower during the initial 30 minutes in the estrogen-treated monkeys compared with both the control and androgen-treated monkeys. These studies suggest that chronically elevated androgen levels in the mature female subhuman primate do not lead to insulin resistance or overt glucose intolerance. (Am J Obstet Gynecol 1987;157:1297-302.)

Key words: Androgens, insulin resistance, primates

Increased circulating androgen concentration in the adult human female has been associated with hyperinsulinemia and insulin resistance as measured by fasting basal insulin levels and the insulin response to an oral or intravenous glucose tolerance test.[1-5] Treatment of women with polycystic ovarian disease with spironolactone lowered both their elevated serum testosterone and basal immunoreactive insulin concentrations, which suggested that androgens may be one factor in the development of insulin resistance.[6] Analysis of factors such as weight and androgen concentrations relative to insulin resistance also supported the role of increased androgen production as a factor in the development of insulin resistance in women.[3] However, hyperandrogenism is often associated with hyperinsulinism caused by genetic disorders of insulin action,

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Table I. Serum gonadal steroid concentrations in monkeys treated long-term with androstenedione or estrone

Steroid	Treatment					
	Control		Androstenedione		Estrone	
	1984 (n = 4)	1985 (n = 4)	1984 (n = 6)	1985 (n = 5)	1984 (n = 4)	1985 (n = 4)
Androstenedione (pg/ml)	1180 ± 206	—	3870 ± 499*	—	1230 ± 185	—
Testosterone (pg/ml)	356 ± 72	346 ± 23	1530 ± 95*	1640 ± 238*	362 ± 65	403 ± 22
Estradiol (pg/ml)	44.3 ± 7.6	39.2 ± 3.5	42.4 ± 4.2	47.4 ± 3.7	78.3 ± 10.4†	69.8 ± 2.5‡

Mean ± SEM.
*$p < 0.01$ compared with controls or estrone-treated monkeys.
†$p < 0.05$ compared with controls or androstenedione-treated monkeys.
‡$p < 0.01$ compared with controls or androstenedione-treated monkeys.

Table II. Basal plasma concentrations of glucose, insulin, and C-peptide in control, androstenedione-, or estrone-treated, fasted female rhesus monkeys

Date (No.)	Treatment					
	Control		Androstenedione		Estrone	
	April 1984 (n = 4)	January 1985 (n = 7)	April 1984 (n = 6)	January 1985 (n = 5)	April 1984 (n = 4)	January 1985 (n = 4)
Glucose (mg/dl)	66.5 ± 6.6*	55.9 ± 2.5	67.6 ± 4.6	59.4 ± 2.6	64.0 ± 5.3	62.3 ± 2.3
Insulin (μU/ml)	56.7 ± 18.9	20.0 ± 2.6	29.6 ± 6.4	30.0 ± 3.9	20.0 ± 5.1	21.8 ± 4.5
C-Peptide (pmol/ml)	0.40 ± 0.15	—	0.25 ± 0.009	—	0.20 ± 0.007	—

Female rhesus monkeys were implanted subcutaneously with silicone rubber tubing that was 4 cm long and packed with estrone or androstenedione or empty (controls). The control and estrone animals and two of the androstenedione animals were started in May 1982, while the remaining four androstenedione-treated animals were first implanted with steroid in July 1980. The tubing with steroid was replaced every 6 to 8 months. Food was withheld for 24 hours before blood samples were obtained during ketamine anesthesia. Two basal samples were obtained 10 to 15 minutes apart and the average of the two values used for calculation of basal concentrations.
*Mean ± SEM.

and therefore it has been suggested that hyperandrogenism in some women might result in part from an action of insulin on the ovarian responsiveness to luteinizing hormone.[7]

One approach to study the role of androgens in insulin resistance in women is to decrease the excessive androgen production[6] or administer antiandrogens[8] and follow the insulin resistance. Studies in obese women with polycystic ovarian disease treated with hypocaloric diets with or without antiandrogenic therapy do not support a major role for androgen in the development of insulin resistance.[8] The interpretation of the results of that study is complicated by the weight loss in combination with the antiandrogen treatment since associated obesity is known to be a major factor in insulin resistance in women with polycystic ovarian disease.[3] Ethinyl estradiol was also administered with the antiandrogen cyproterone acetate. The combination of these two factors, weight loss and estrogen, might mask the role of androgens in insulin resistance in women with polycystic ovarian disease.

An alternative approach is to establish a hyperandrogen condition in the absence of obesity and measure basal insulin levels and the insulin response to a glucose bolus. The present study reports the results of the comparison of basal insulin levels and subsequent insulin response to a bolus of glucose in female monkeys that have elevated circulating androstenedione and testosterone, produced by silicone rubber implants packed with crystalline androstenedione for 2 to 4½ years. The results are compared with those of female monkeys with chronic exposure to elevated estrone and estradiol levels. These monkeys are part of an ongoing investigation to study the effect of chronically elevated gonadal steroids on the endocrinology and physiology of female primates.[9]

Material and methods

Adult female rhesus monkeys with regular menstrual cycles were caged individually in a room at a controlled temperature of 22° C and with 12-hour light to 12-hour dark cycles. The hormonal treatment and weight of

these animals have been previously reported.[9] For the present study, three additional nonobese female monkeys from the colony with regular menstrual cycles were added to the control group. At the time of the January 1985 test, the weight of the animals were: controls, 4.9, 5.1, 5.5, 5.6, 5.6, 6.0, and 6.0 kg; androstenedione-treated, 5.0, 5.4, 5.4, 6.7, and 7.6 kg; and estrogen-treated, 3.3, 5.1, 5.7, and 6.4 kg. The animals received Ralston-Purina high-protein chow diet 5045 once daily and water ad libitum and were given fresh fruit twice weekly. All food and wood shavings were removed approximately 24 hours before blood samples were taken for basal values and an intravenous glucose bolus was given. Monkeys used in these studies were maintained in accordance with the guidelines of the Committee on Animals of the University of Pittsburgh School of Medicine and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources (1978 Department of Health, Education and Welfare publication National Institutes of Health 78-23, National Research Council).

Serum androstenedione concentrations were determined by a modification of the radioimmunoassay (RIA) method previously described[9] with methylene dichloride substituted for ether for extraction of the serum samples, and Celite column chromatography replaced paper chromatography for the purification. The androstenedione antiserum was purchased from Hazelton Laboratories American (Vienna, Va.). Serum testosterone and estradiol concentrations were determined by the same assays as reported.[9]

Plasma glucose concentrations were determined by the glucose oxidase method, insulin by the human double antibody RIA method,[10] and C-peptide by a single antibody RIA method for human C-peptide (Serono RIA kit, Serono Diagnostics, Inc., Braintree, Mass.). In the insulin assay the mean difference of samples assayed in duplicate in the same assay was 2.5%. The mean difference of 19 samples assayed in two separate assays was 7.4% ± 6.5% (SD).

After 24 hours of fasting, the monkeys were anesthetized with ketamine, and a catheter was inserted into the antecubital or femoral vein for the withdrawal of blood samples or the administration of a bolus of glucose in saline solution (0.5 gm/kg body weight). After two blood samples were obtained 10 to 15 minutes apart for the determination of basal glucose, insulin, and C-peptide concentrations, the glucose was given as a bolus over 30 seconds, followed by a flush of saline solution. After completion of the glucose injection, 3 ml blood samples were then taken in heparinized syringes at 2, 5, 10, 20, 30, 40, and 50 minutes in the January 1985 study and an additional 60-minute sample in the April 1984 study. The blood was centrifuged at 4° C, and the plasma was stored at −60° C for glucose, insulin, and C-peptide analyses. Statistical analyses were by one-way analysis of variance and *t* test for multiple comparison.[11] The data of the April 1984 and January 1985 studies were analyzed separately and not pooled together. The variance between the standard deviations of each analysis between groups was tested by the Barlett test.[11]

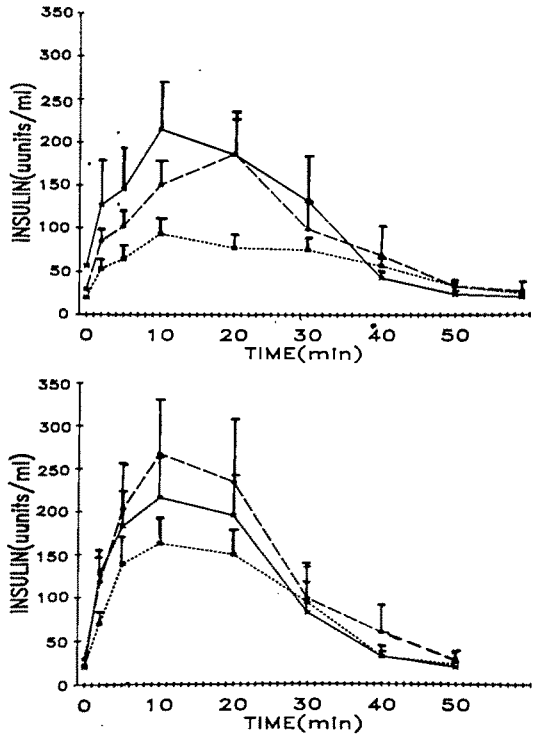


Fig. 1. Insulin responses to an intravenous bolus of glucose in fasted control, androstenedione-, or estrone-treated female rhesus monkeys. After a 24-hour fast and blood samples were obtained for basal plasma concentrations, each monkey was given an intravenous bolus of glucose in saline solution (0.5 gm/kg body weight), and blood samples were collected at time periods indicated. Hormonal treatment was as described in the legend of Table I. The number of animals studied in each group in April 1984 and January 1985 is listed in Table II. *Top panel,* results of the test done in April 1984. *Bottom panel,* January 1985 (see Methods section). (X——X = Controls; o - - - - o = androstenedione-treated; □ · · · · □ = estrone-treated.)

Results

Serum concentrations of testosterone, androstenedione, and estradiol in the monkeys during the early follicular phase or periods of anovulation were in the same range as previously reported[9] (Table I). Although not measured at the time of the insulin studies, previous studies indicated that the estrone capsules results in serum concentrations of estrone of approximately 150 pg/ml compared with estrone concentrations of 65 pg/ml in the control or androstenedione-treated monkeys.[9]

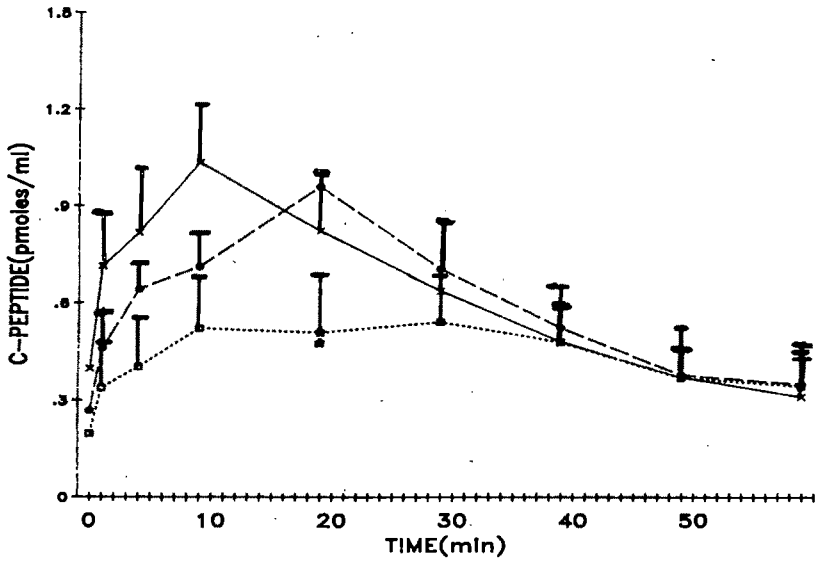


Fig. 2. C-peptide response to an intravenous bolus of glucose in fasted control, androstenedione-, or estrone-treated female rhesus monkeys. The study is that described in the legend of Fig. 1 and Tables II and III (April 1984). The number of animals studied is listed in Table II. (*$p < 0.01$ compared with maximal response of control [X——X] or androstenedione [o - - - - o] animals.)

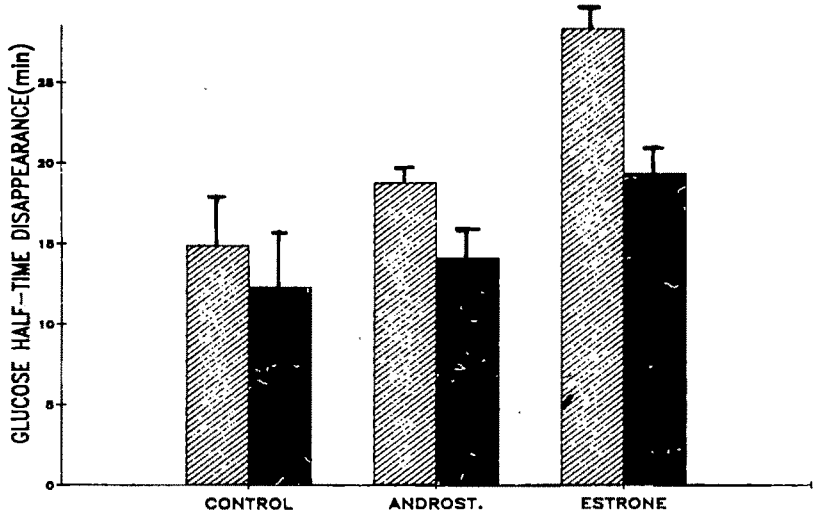


Fig. 3. The half-time (t-½) disappearance rate of glucose after an intravenous glucose bolus to fasted control, androstenedione-, and estrone-treated female rhesus monkeys. The study is described in the legends of Fig. 1 and Tables II and III. The number of animals studied in each group in April 1984 and January 1985 is listed in Table II. Plasma glucose concentrations were converted to their ln concentrations, and t-½ was calculated from least squares analysis of the disappearance rates. (▨ = Study done in April 1984; ■ = repeat study done in January 1985.)

Chronic and continuous elevation of circulating levels of androgens or estrogens did not significantly increase the basal concentrations of plasma glucose, insulin, or C-peptide after a 24-hour fast (Table II). Although the basal levels of insulin tended to be highest in the androgen-treated female monkeys compared with control (January 1985) and the estrogen-treated monkeys, they were not significantly different and returned to similar basal concentrations in all three groups 50 minutes after the administration of an intravenous bolus of glucose (Fig. 1). One of the four control animals had an unexplained high basal concentration of insulin in April 1984 that was not repeated in January 1985, at which time it was in the normal range (Table II).

The insulin-response to an intravenous bolus injec-

Table III. Intravenous glucose tolerance test in 24-hour fasted female monkeys chronically treated with androstenedione or estrone

	Treatment					
	Control		*Androstenedione*		*Estrone*	
	April 1984 (n = 4)	*January 1985 (n = 7)*	*April 1984 (n = 6)*	*January 1985 (n = 5)*	*April 1984 (n = 4)*	*January 1985 (n = 4)*
Total glucose* (mg × 10^{-3})	2.7 ± 0.22	2.7 ± 0.19	3.2 ± 0.19	3.1 ± 0.41	3.4 ± 0.25	3.2 ± 0.36
Total insulin* (μU/ml × 10^{-3})	3.5 ± 0.65	4.5 ± 1.0	3.2 ± 0.76	5.2 ± 1.6	1.7 ± 0.31	3.3 ± 0.32
Total C-peptide* (pmol/ml)	13.9 ± 2.6	—	14.6 ± 1.2	—	8.3 ± 2.0	—
Total insulin/glucose	1.3 ± 0.22	1.7 ± 0.39	0.97 ± 0.17	1.7 ± 0.46	0.43 ± 0.006‡	1.1 ± 0.16
Ratio of maximal insulin/C-peptide†	226 ± 28	—	216 ± 42	—	216 ± 59	—

*After basal plasma samples were obtained, the monkeys were given an intravenous bolus of 0.5 gm glucose/kg body weight over 30 seconds and blood samples were collected at 2, 5, 10, 20, 30, 40, and 50 minutes in the January 1985 study and an additional sample at 60 minutes in the April 1984 study. Basal values were subtracted from the concentrations obtained between 2 and 30 minutes, the values were plotted on graph paper, and the area under the curves was integrated by a "smart pad" and software program Lotus 1, 2, and 3.

†The ratio of maximal insulin/C-peptide concentrations was obtained from the data of Figs. 2 and 3.

‡$p < 0.05$ compared with control and androstenedione-treated animals.

tion of glucose was tested in the control, androgen-, and estrogen-treated female monkeys (Figs. 1 and 2, and Table III). The maximal glucose response and cumulative plasma glucose concentration were not different in all three groups in both test periods. However, the glucose disappearance rate (i.e., t-½, Fig. 3) was significantly slower for the time interval of 2 to 30 minutes in the estrogen-treated monkeys in both test periods. (Plasma glucose concentrations returned to basal levels at 30 or 40 minutes after injection.) Although the insulin response to the glucose bolus was not statistically different among the three groups, the insulin response tended to be less in the estrone-treated female monkeys in both test periods (Fig. 1). The plasma concentrations of insulin and C-peptide in response to the intravenous glucose bolus were similar among the three groups (Table III), as were the calculated mean ratios of the total insulin/C-peptide responses: 252, 219, and 205. Although the insulin responses were not different among the three groups, the response differed in that the variance in the plasma insulin concentrations of the estrone-treated monkeys was significantly less than that of the control or androstenedione-treated monkeys. Thus the estrone-treated animals had a more uniform insulin response to the intravenous glucose bolus than either the control or androgen-treated animals. Because of the difference in the variance of the estrone group compared with the control and androstenedione-treated animals, the insulin response (Table III) was also analyzed by the Kruskal-Wallis test. There was no significant difference between groups in either the April 1984 ($p = 0.05$ to 0.1) or January 1985 ($p > 0.1$) insulin response.

The insulin/glucose (area) ratios of the estrogen-treated animals were significantly less in one test (April 1984) compared with either control or androgen-treated animals and tended to be lower in the second test (January 1985) but was not significantly different.

Comment

During the past several years, considerable attention has been focused on the associated increase of androgen production in women and the development of insulin resistance.[1-8] It is unclear whether increased androgen production can lead to insulin resistance, whether increased circulating insulin enhances luteinizing hormone stimulation of ovarian androgen secretion,[7, 8] or whether the two phenomena are associated with an additional, unknown metabolic parameter(s). The present studies examined the question of the effect of increased circulating androgens on basal insulin concentrations and the insulin response to intravenous glucose in a primate model where the androgen concentrations have been chronically elevated by means of an androstenedione silicone rubber implant. Androstenedione and testosterone serum concentrations were three to four times higher than that present in control or estrone-treated female monkeys (Table I). Since it has been reported that the intravenous glucose tolerance test in monkeys is variable,[12] the glucose tolerance test was repeated twice in the same animals at an interval of 10 months. The intravenous glucose-tolerance curve and insulin response of the control andandrogen-treated monkeys were similar to those reported for a larger number of normal rhesus monkeys.[13] The results indicate that a chronic elevation of the circulating concentrations of androstenedione and testosterone for 2 to 4½ years does not alter either the basal glucose or insulin concentrations or the glucose disappearance or insulin response to an intravenous bolus of glucose in

fasted monkeys. If insulin secretion and its peripheral actions are modulated in a similar manner in the rhesus monkey as in women, the present results suggest that the reported insulin resistance in women with hyperandrogenism may not be a result of hyperandrogenism alone. A similar conclusion has recently been reported in a study of antiandrogens and insulin resistance in obese women with polycystic ovarian disease.[8]

Monkeys chronically exposed to elevated estrone and estradiol had a lower insulin response to the intravenous glucose bolus. C-peptide concentrations paralleled the insulin response. Although the insulin and C-peptide concentrations of the estrone-treated monkeys were not significantly different from the corresponding concentrations in control and androstenedione-treated animals, a lower insulin response to glucose was observed in both test periods. The glucose disappearance rate was significantly slower in both test periods in the estrone-treated monkeys compared with either the control or androgen-treated monkeys. Furthermore, the ratio of insulin area to glucose area was lowest for the estrone-treated animals in both test periods. These results suggest that chronic elevation of the estrone and estradiol concentrations may modulate the pancreatic response to glucose, even though the estrone and estradiol concentrations were less than three times higher than in the early follicular phase[9] (Table I).

It is difficult to compare the observed estrogen effect in the monkey with similar studies in humans or monkeys, since (1) different estrogen preparations were used, (2) the dosages were usually higher and circulating estrogen concentrations were not measured, and (3) the duration of such studies was usually for shorter time periods. Nevertheless, the present results were not anticipated since estrogens have been observed to have either no effect or a stimulatory effect on the insulin response to glucose.[12, 14] The smaller variation in insulin and C-peptide concentrations in the estrone-treated animals in response to glucose might provide the explanation. Insulin responses to glucose are dependent on the prior metabolic history of the animal.[15] It may be that monkeys chronically treated with estrogen have a more consistent dietary pattern than that of cycling control or androgen-treated animals,[16, 17] resulting in a more consistent insulin response among the estrogen treatment group.

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A new look at uterine muscle contraction

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Recent progress in our understanding of uterine smooth muscle contraction is reviewed. We no longer believe that actin-myosin interaction in the myometrium occurs through activation of the thin filament; but it is triggered by calcium-dependent phosphorylation of myosin in the thick filament. Calcium is now thought to originate from both extracellular and intracellular sources. Calcium can enter the cell through either a voltage- or a hormone-controlled calcium channel. The intracellular source of calcium is the sarcoplasmic reticulum. The effect of oxytocin in human labor is no longer considered the result of increased circulating oxytocin but rather of increased oxytocin receptors. In contrast, the contractile action of some prostaglandins is related to increased prostaglandin formation at human parturition. The step between hormone binding and cellular action is mediated by second messengers. The uterine-relaxing action of cyclic adenosine monophosphate is now thought to be limited to the inhibition of myosin phosphorylation. Recently discovered second messengers for contraction of the myometrium are phosphoinositides; their turnover causes calcium release from the sarcoplasmic reticulum. Guanine nucleotides are thought to be modulators of these two second messengers. (Am J Obstet Gynecol 1987;157:1303-15.)

The past decade has seen the following three new developments contribute to our understanding of smooth muscle contraction and in particular to uterine muscle contraction: (1) The actin-myosin interaction in uterine smooth muscle was shown to be different from that in skeletal muscle; (2) the dominant role of intracellular calcium in smooth muscle contraction was firmly established; and (3) a new second messenger, inositol trisphosphate, was discovered. The common denominator for these reactions is calcium. In this review, recent findings will be discussed, and molecular mechanisms will be reviewed as far as they are known. Since not all experimental data are available for the myometrium, at times we will have to refer to other smooth muscles. This is with some misgivings because where detailed studies have been done, differences have been found, even between arteries from various locations. Nevertheless, we have made progress in our understanding of normal events in myometrial contraction.

Contraction

Smooth muscle structure. While it had been known for many years that actin and myosin were present in smooth muscle, it was thought that smooth muscle contraction was similar to that of skeletal muscle, which is represented by the sliding filament model. Arrays of thick protein filaments containing the protein myosin interdigitate with arrays of thin filaments containing the protein actin. In contraction the filaments move past each other because of actin-myosin interaction without a change in length of the individual filament, but the muscle as a whole changes its length. Interaction of actin and myosin occurs through the myosin cross-bridges, protrusions from the myosin filament that reversibly bind to actin. The energy for contraction comes from adenosine triphosphate (ATP) hydrolysis. In skeletal muscle the interaction of actin and myosin is inhibited by the presence of the tropomyosin-troponin complex in the thin filament. The inhibition is removed when troponin binds calcium, which causes a steric shift of the complex. Attempts to visualize thick filaments in smooth muscle were unsuccessful for many years. This was partly from technical difficulties and partly from the lower myosin content of smooth muscle.[1] In smooth muscle, the alignment of the filaments is less regular; hence no cross-striations are observed. The thin filaments can be arranged in parallel with the cell axis, in arrays of various shapes, and in rosettes when in association with myosin, or at random.[2, 3] The actin filaments connect through dense bodies with the plasma membrane. This modification allows for many features characteristic of smooth muscle—its ability to shorten to less than 50% and lengthen to more than twice its resting length, greater force, and low velocity of contraction.

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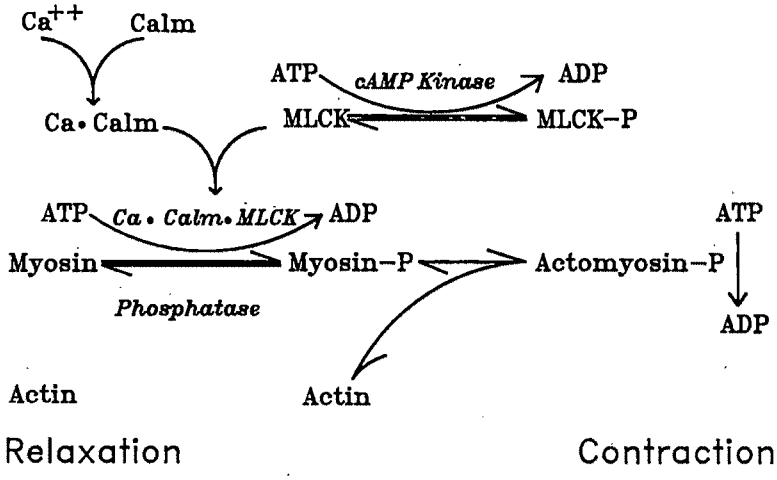


Fig. 1. Diagram of contraction-relaxation cycle of smooth muscle. (*Calm* = Calmodulin; *cAMP kinase* = cAMP-dependent protein kinase; *MLCK* = myosin light-chain kinase; *MLCK-P* = phosphorylated myosin light-chain kinase; *Myosin-P* = phosphorylated myosin; *Actomyosin-P* = phosphorylated actomyosin; *ADP* = adenosinediphosphate; *ATP* = adenosine triphosphate.)

The actin-myosin interaction in smooth muscle. In smooth muscle, most of the evidence indicates that troponin plays only a minor role, if any, in the contraction-relaxation cycle. Myosin consists of four polypeptide chains, two heavy chains and two light chains. In smooth muscle contraction, the 20,000 molecular weight myosin light chain is phosphorylated by the enzyme myosin light-chain kinase when the calcium concentration rises. Only when phosphorylated can the myosin light chain interact with actin. The reaction requires the protein calmodulin; the calcium-calmodulin complex then binds to myosin light-chain kinase for activation. When the calcium level decreases, the calmodulin dissociates from the myosin light-chain kinase and the myosin light-chain kinase becomes inactive. Dephosphorylation of the myosin light chain occurs at a steady rate by a phosphatase.[4, 5] This reaction has been demonstrated in vitro and in intact smooth muscle.[6] The activity of the myosin light-chain kinase is modulated by a cyclic adenosine monophosphate (cAMP)–dependent protein kinase. This protein kinase catalyzes phosphorylation of myosin light-chain kinase.[7] The phosphorylated myosin light-chain kinase has only a weak affinity for the calcium calmodulin complex, thus becoming inactive. Dephosphorylation of myosin and relaxation follow.[6, 8] This reaction sequence is illustrated in Fig. 1.

In the myometrium, myosin light-chain phosphorylation and dephosphorylation were demonstrated for the contraction-relaxation cycle.[9] Serine and threonine residues of the myosin light chain were phosphorylated.[10]

Less popular hypotheses, which may well play a role as auxiliary mechanisms for smooth muscle contraction, focus on calcium stimulation acting on the thin filament. One hypothesis involves the binding of the protein leiotonin to actin[11], the other, the calcium-dependent regulation by the protein caldesmon, present in the thin filaments.[2] No attempts have been made so far to demonstrate these proteins in the myometrium.

Pharmacomechanical coupling

The events leading to muscle contraction start at the cell membrane with a stimulus. The stimulus initiates an action potential, which is followed by a contraction. This is called electromechanical coupling and is a means of excitation-contraction coupling in skeletal muscle. It has been found that in smooth muscle, electromechanical coupling does not fully explain observed phenomena.[12] In smooth muscle, there is not always an action potential. There can be either electrical or hormonal stimulation. Hormone binding to a receptor at the cell surface can activate a chain of events that ultimately lead to muscle contraction. This is called pharmacomechanical coupling.[13] The search for its mechanism and some of the answers are discussed in the following sections.

The role of calcium

The smooth muscle cell membrane. A diagram of the smooth muscle cell is shown in Fig. 2, and the actin and myosin filaments are seen. At 10^{-8} to 10^{-7} M calcium, the muscle is relaxed, and at 10^{-6} M calcium, the filaments interact and the muscle contracts without changing filament length. From where does the calcium for contraction come? It has long been known that part of the calcium comes from outside the cell. The calcium concentration outside the cell in the interstitial fluid is 10^{-3} M, that is, 10,000-fold higher than that inside the cell. Although there is an electrochemical gradient, calcium does not rush into the cell at rest because of the

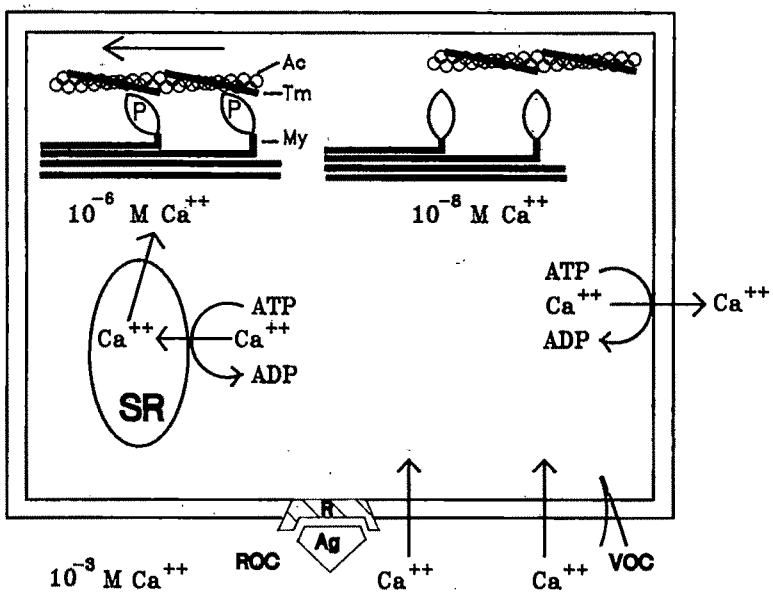


Fig. 2. Calcium sources for myometrial contraction. On top, sliding filament of muscle contraction *(left)* and relaxation *(right)*. (*Ac* = Actin; *Tm* = tropomyosin; *My* = myosin; *P-* = phosphorylated; *SR* = sarcoplasmic reticulum; *R* = receptor; *Ag* = agonist; *ROC* = receptor-operated channel; *VOC* = voltage-operated channel; *ATP* = adenosine triphosphate; *ADP* = adenosine diphosphate.)

low calcium permeability of the cell membrane. Calcium can enter the cell through an ion channel with an action potential. Calcium binds reversibly to a specific site at or near the surface of the channel. Subsequent movement of the calcium ion is determined by current flow and the extracellular concentration of calcium.

Calcium channels. Some chemical agents that inhibit contraction have been found to directly block calcium entry. They are called calcium channel blockers, or calcium entry blockers; they bind to the cell membrane within the membrane calcium channel.[14] They make up a wide range of structures ranging from inorganic ions such as lanthanum to some organic compounds. The more interesting ones are the dihydropyridines, nitrendipine, nifedipine, nicardipine, and other unrelated compounds such as diltiazem, verapamil, and D 600. Some of the latter have been found to have additional action in the myometrium.[15] Observations on muscle strips from the parturient rat uterus showed that D 600 and other calcium entry blockers abolished spontaneous contractions and reduced the force of electrically evoked contractions. However, D 600 did not block the tonic contraction evoked by prostaglandin (PG) $F_{2\alpha}$.[16, 17] These experiments suggested the presence of another type of calcium channel, namely, one controlled by hormones. It was subsequently shown in rabbit aorta that the amount of calcium influx on maximal stimulation by potassium or norepinephrine was dissimilar and each was less than the calcium influx when both were applied simultaneously. This led to the suggestion that an additional set of calcium channels could be opened by norepinephrine after maximal calcium influx caused by potassium.[18] Furthermore, D 600 preferentially inhibited potassium-activated channels, and in general, higher concentrations of calcium entry blockers are required to block the receptor-operated channel than the voltage-operated channel. Clinically, calcium channel blockers produce adequate relaxation and have been used to inhibit spontaneous contractions in premature labor[19-21] and myometrial hyperactivity in primary dysmenorrhea.[22-24]

Calcium efflux through the cell membrane. The calcium that entered the cell for contraction must eventually be pumped out of the cell. The energy requiring transport of calcium across membranes is mediated by enzymes, the so-called calcium and magnesium adenosine triphosphatases (Ca,Mg-ATPase), because these enzymes split adenosine triphosphate (ATP) and require calcium and magnesium for their activation. The ATPase of the cell membrane is stimulated by the protein calmodulin[25] and inhibited by the chemical vanadate.[26]

In cardiac muscle an important mechanism for moving calcium across the cell membrane is Na^+-Ca^{++} exchange. This mechanism works by removing calcium from the inside of the cell in exchange for incoming sodium. The Na^+-Ca^{++} exchange has been observed in some smooth muscles including the uterus.[27-30] However, the Na^+-Ca^{++} exchange appears to be of low magnitude in smooth muscle, which may be from technical difficulties in experimentation. Its physiologic significance is debated.[18]

Role of the sarcoplasmic reticulum. Recent observations have shown that the influx of calcium with an action potential is insufficient to activate fully the contractile mechanism and sustain maximum contraction

of smooth muscle.[31] Much of the required calcium comes from intracellular stores, but there are quantitative differences between various smooth muscles.

In skeletal muscle there is an abundant system of intracellular channels or tubules, called the sarcoplasmic reticulum. This system stores calcium in relaxation. On an impulse conducted from the cell membrane, this calcium is rapidly released during contraction. This mechanism is useful because of the large size of the skeletal muscle cell and the speed of contraction. In contrast, the smooth muscle cell is much smaller than the skeletal muscle cell and contractions are slow. Hence the role of the sarcoplasmic reticulum in smooth muscle contraction was questioned. Early observations demonstrated a less abundant sarcoplasmic reticulum in smooth muscle cells.[32, 33] However, recent studies with the electron microscope have shown a sarcoplasmic reticulum similar to that in skeletal muscle.[34] The sarcoplasmic reticulum is estimated to occupy about 1.5% to 7.5% of the cell volume of the smooth muscle cell and is particularly abundant in some blood vessels and in pregnant or estrogen-treated uterus.[34] It has been demonstrated that in vascular smooth muscle, in calcium-free solution, and in the presence of lanthanum, which blocks passage of calcium through the cell membrane, intracellular stores of calcium are sufficient to elicit maximal contraction through recycling of intracellular calcium stores.[35] Ultrastructural studies that use electron-probe analysis have shown high calcium concentrations in the sarcoplasmic reticulum of relaxed smooth muscles. From these findings it was concluded that the sarcoplasmic reticulum and not the cell membrane is the organelle primarily responsible for the physiologic regulation of the cytoplasmic calcium.[31] This hypothesis is also borne out by physiologic and biochemical observations. It is presently thought that mitochondria play no role in the regulation of cytoplasmic calcium at physiologic levels.[37]

In relaxation calcium is sequestered by the sarcoplasmic reticulum. This mechanism for reducing the intracellular calcium concentration is in addition to the extrusion of calcium through the plasma membrane previously discussed.

Calcium uptake into the sarcoplasmic reticulum. Calcium uptake into the sarcoplasmic reticulum will be discussed first. More is known about calcium uptake than about calcium release, since the former has been easier to study.

The transport of calcium into the sarcoplasmic reticulum can best be demonstrated on the subcellular level in microsomal preparations. The techniques for isolating microsomal preparations are based on the procedures used for the preparation of the sarcoplasmic reticulum of skeletal muscle. They were modified because of the large amount of connective tissue in smooth muscle that prevents easy isolation of smooth muscle components. Furthermore, smooth muscle contains much more cell membrane than does skeletal muscle because of the smaller size of the smooth muscle cell. Thus it is harder to prepare pure sarcoplasmic reticulum from the smooth muscle cell. The degree of purity of the preparation is difficult to document because there are no unique markers.[38, 39]

In our laboratory we have isolated sarcoplasmic reticulum from term pregnant bovine myometrium and human uteri obtained at cesarean hysterectomy.[40-42] Under the electron microscope our sarcoplasmic reticulum preparation shows vesicles of about 1000 Å (100 nm) diameter.[40] In the presence of ATP and calcium, the sarcoplasmic reticulum preparations accumulate calcium on the inside of the vesicles, that is, they take up calcium from solution. The calcium uptake increases with an increased amount of calcium in solution[43] and is stimulated by oxalate. The process is temperature-dependent and ATP is required. This in vitro process of calcium removal from solution and transport into a storage area would cause in vivo muscle relaxation.

Adenosine triphosphatase. Parallel with calcium uptake is ATPase activity. A Ca,Mg-ATPase in the sarcoplasmic reticulum membrane breaks down ATP to adenosine diphosphate (ADP) by removing the terminal phosphate group.[12] This Ca,Mg-ATPase has different characteristics from the ATPase of the cell membrane. These characteristics serve to identify the origin of the isolated microsomal preparations. In contrast to the ATPase of the cell membrane, the ATPase of the sarcoplasmic reticulum is not sensitive to stimulation by calmodulin or inhibition by vanadate.[25, 26, 44] On the molecular level the ATPases of sarcoplasmic reticulum and cell membrane differ in molecular weight (sarcoplasmic reticulum 100,000 to 110,000; cell membrane 130,000) as shown by electrophoresis of the phosphorylated intermediate.[45, 46] With tryptic digestion, little difference could be found between the molecular weight of the ATPase of aortic smooth muscle and that of skeletal muscle. However, there are immunologic differences.[47] The tryptic digestion pattern of the ATPase of the sarcoplasmic reticulum is different from that of the cell membrane ATPase.[47]

Mechanism of calcium transport. To understand the events that occur in the transport of calcium through the membranes of the sarcoplasmic reticulum, it is advantageous to examine partial reactions, since these can be more easily interpreted. Several steps in the cycle of calcium uptake into sarcoplasmic reticulum vesicles and calcium release and the mechanism of action of the Ca,Mg-ATPase have been recently elucidated.

The first step in calcium transport is the binding of calcium to the outside of the vesicular membrane of sarcoplasmic reticulum. Two types of calcium binding sites were found with association constants of 8 and 0.1 ($\times 10^6\ M^{-1}$). The number of binding sites was four and 10 nmol/mg of protein, respectively. The high-affinity binding site appears to be part of the transport ATPase;

the presence of a second binding site may indicate that calcium serves an additional function.[48]

The use of magnesium sulfate as a tocolytic agent in the treatment of premature labor[49-51] is well known. It has been shown that Mg^{++} abolishes contractions in uterine muscle strips.[52] In the presence of 5 mM Mg^{++}, the maximum number of low-affinity calcium sites was reduced to half, whereas the high-affinity calcium binding sites were not affected by Mg^{++}.[48] Whether the changes observed in vitro correlate with the in vivo action of Mg^{++} remains to be documented.

The next step in calcium transport is the formation of a high-energy intermediate in the presence of ATP and calcium. This is demonstrated by showing a phosphorylated ATPase. Using [^{32}P]ATP, we obtained results that document phosphorylation of the ATPase,[43] which were consistent with the following scheme, modeled after that presently established for calcium transport in skeletal muscle.

$$Ca_{out} + E + ATP \rightleftharpoons Ca \cdot E \cdot P + ADP$$
$$Ca \cdot E \cdot P \rightleftharpoons E + P + Ca_{in}$$

where E is the 110,000 molecular weight ATPase enzyme. E binds calcium plus ATP with activation of the enzyme. The formation of an intermediate, Ca · E · P, results from the binding of a terminal phosphate, P, of ATP to the aspartyl residue of the ATPase. The ATP is split, ADP is released, calcium is then transported to the inside of the vesicles, and phosphate is released.

Calcium release. The reverse process, the release of calcium from the sarcoplasmic reticulum vesicles, does not require energy and leads in vivo to contraction. Experiments with microsomal vesicles have shown that the vesicles are relatively impermeable to calcium with a half time of 100 minutes at 37° C. This means that it will take 100 minutes for half the accumulated calcium to diffuse out of the vesicles. However, this can be speeded up by use of an ionophore. Ionophores move calcium through a membrane down the concentration gradient. The release of calcium with an inophore shows that the calcium is inside the vesicles[53]; however, this is not a physiologic mechanism. Therefore we are left with the question, what is the physiologic mechanism for calcium release?

Hormonal calcium mobilization. The uptake and release of calcium can be inhibited or enhanced by various agents. As shown in partially purified microsomal preparations in our laboratory, the uterine contractile prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) inhibited calcium uptake and enhanced calcium release; oxytocin also inhibited calcium uptake. Consistent with its pharmacologic action, PGE_2 was more potent than $PGF_{2\alpha}$. Much greater effects of oxytocin and slightly greater effects of $PGF_{2\alpha}$ were observed in microsomes derived from pregnant uteri than in those from nonpregnant uteri.[41, 42, 54, 55] The increase in free calcium is a prerequisite for contraction. Similar effects of $PGF_{2\alpha}$ were recently observed in vascular smooth muscle cells.[56] The release of calcium from intracellular calcium stores by prostaglandins is further supported by experiments showing prostaglandin-induced contraction of rat uterine horns in calcium-free medium.[57] It is unclear whether this is a direct effect of prostaglandins or whether it is mediated through a messenger.

Hormonal stimulation and hormone receptors

The two physiologic hormones that cause uterine contractions are oxytocin and prostaglandin. Oxytocin is the preferred pharmacologic agent for induction of labor because of its high specificity in contracting the uterus. However, the physiologic role of these two hormones in the onset of human labor is controversial, and the picture is muddled by great differences between species. In the context of this discussion, it is unnecessary to go into arguments of which is the more important.

Oxytocin. Some workers have shown a rising trend in oxytocin concentration in the maternal plasma with gestation. This trend increased toward the end of pregnancy.[58, 59] However, technical difficulties with the assay, fluctuating patterns, and wide scatter of obtained oxytocin values make these results questionable. More recent work showed no rise in plasma oxytocin concentration until late in the second stage of labor.[60, 61]

An alternative explanation for the effect of oxytocin would be a rise in oxytocin receptors. This is manifested by increased sensitivity to oxytocin. Increased sensitivity of the myometrium to oxytocin with advancing pregnancy, especially toward the very end of pregnancy, has long been known.[62] Indeed, it was shown that the sensitivity to oxytocin correlated with preterm and postterm delivery, with women who delivered prematurely having the highest sensitivity to oxytocin.[63]

Specific receptors for oxytocin have been identified in the rat,[64, 65] ewe,[66] rabbit,[67] and human uterus.[68, 69] In binding experiments with purified subcellular membrane fractions, the oxytocin receptors were found to be located in the myometrial cell membrane. The dissociation constant was calculated to be in the range of 2 to 3 nM.[64, 66, 67] The concentration of oxytocin receptors rises toward the end of pregnancy, is maximal during labor, and then declines.[64] In subcellular preparations from a human uterus, there was also an increase in oxytocin receptor concentration with gestational age, as well as some increase in binding affinity at term.[68] Similar changes in oxytocin receptor concentration were found in human uteri at various stages of gestation and in labor, with lower values before the onset of labor and in failed induction of labor with oxytocin.[69] Furthermore, the concentration of oxytocin receptors appears to be regulated by estrogen and progesterone, at least in the rat and rabbit, with estrogen increasing the number of oxytocin receptors and progesterone inhib-

iting this increase.[64, 70, 71] These findings correlate with observed physiologic function and suggest that the increase in uterine sensitivity to oxytocin is caused by an increase in myometrial oxytocin binding sites.

Prostaglandins. Unlike oxytocin, which is a circulating hormone, prostaglandins originate at or near their site of action, that is, in the endometrial and myometrial cell. Therefore one would expect differences in the physiologic action of these two hormones. Early work indicated that administration of PGE_2 and $PGF_{2\alpha}$ and prostaglandin analogs by various routes causes contraction of the pregnant and nonpregnant uterus and can initiate labor or abortion at any stage of gestation.[72, 73]

Prostaglandins play an important role in both the nonpregnant and pregnant uterus as evidenced by physiologic, pathologic, and pharmacologic findings. During the menstrual cycle, high concentrations of both PGE and PGF were found in the human myometrium during the late proliferative phase, coinciding with ovulation. After ovulation the concentration of PGE gradually declined and stayed low, whereas the PGF concentration showed a second peak during the late secretory phase, approximately coinciding with the onset of menstruation.[74] However, in humans and primates, prostaglandins have no luteolytic effect, in contrast to domestic animals.[75, 76] High levels of $PGF_{2\alpha}$ and PGE were found to be present in menstrual fluid collected from women suffering from primary dysmenorrhea.[77] Prostaglandin synthesis inhibitors can stop menstrual cramps and pain in primary dysmenorrhea.[78]

Prostaglandins are detected by radioimmunoassay in the amniotic fluid at about midpregnancy, and their concentration rises through the remainder of pregnancy and into spontaneous labor, with PGE_2 levels higher than those of $PGF_{2\alpha}$.[79] Their concentration in the amniotic fluid, as well as that of arachidonic acid, are higher during labor than before labor and increase with labor.[80] The levels of PGF were found to be higher in women going into spontaneous labor than in women requiring oxytocin induction.[81, 82] However, measurement of prostaglandins in the amniotic fluid is influenced by the mode of obtaining the samples; results are higher in samples collected at amniotomy than in those obtained by amniocentesis.[83]

The measurement of prostaglandins in peripheral plasma is even more difficult than that in amniotic fluid because of their low concentration, the ability of blood platelets to synthesize prostaglandins, and the destruction of prostaglandins by one passage through the lungs.[84] More reliable measurements are obtained by monitoring a prostaglandin metabolite, 13,14-dihydro-15-keto-PGF, which is present in much higher concentration and not synthesized in platelets. With these measurements no increase in prostaglandin concentration in the peripheral blood was noted up to the onset of labor.[85] On the other hand, 13,14-dihydro-15-keto-PGF levels have been found to rise concomitant with cervical ripening without uterine activity before the onset of labor.[86] Taken together these findings lead to the concept that increased synthesis of prostaglandins plays a dominant role in human parturition.

The events that lead to the formation of prostaglandins in human parturition begin in the fetal membranes (amnion and chorion) and also in the decidua. Prostaglandin production is greatest in the amnion, followed by the chorion, and finally the decidua.[87] The first step is the release of free arachidonic acid. Arachidonic acid is the obligatory precursor of the prostaglandins of the "2" series. Arachidonic acid is not stored in human tissues, but human fetal membranes accumulate arachidonic acid in esterified form in the form of glycerophospholipids. The major pathway of arachidonic acid release is by phospholipase A_2 action. This enzyme breaks down glycerophospholipids in the "2" position. The end products of hydrolysis are free fatty acid and lysoglycerophospholipid. Lysoglycerophospholipids are cytolytic agents. They break down cell membranes and also lysosomal membranes inside the cell. Lysosomes contain more phospholipase. Its release causes accelerated release of arachidonic acid and thus accelerated prostaglandin synthesis from preformed glycerophospholipids in the fetal membranes. The preferred substrate for phospholipase A_2 in the fetal membranes is phosphatidyl ethanolamine, which has arachidonic acid in the "2" position.[80, 88, 89] PGE_2 and $PGF_{2\alpha}$ are subsequently formed from arachidonic acid in a multistep process, the first step of which is catalyzed by the enzyme cyclooxygenase.[84] The intermediate endoperoxide and thromboxane also contract the uterus. A secondary pathway of arachidonic acid mobilization starts from phosphatidylinositol. Its breakdown requires three enzymes, phospholipase C, diacylglycerol lipase, and monoacylglycerol lipase.[89, 90]

The stimulus for increased prostaglandin formation in the fetal membranes at the onset of human parturition may be estrogens.[91] Estradiol was shown to stimulate cyclooxygenase activity in the rat uterus[92, 93] and aortic smooth muscle cells.[94] Stimulation of prostaglandin synthesis by oxytocin has been observed in decidua parietalis but not in decidua vera from the membranes or the myometrium.[95, 96] There is some evidence that calcium plays a role in arachidonic acid mobilization followed by prostaglandin production in amnion tissue.[89]

There is a striking association between premature labor and intrauterine, endocervical, and urinary tract infection. It has been shown that many of the organisms that cause these infections have phospholipase A_2 activity.[97] This would lead to increased release of arachidonic acid from preformed glycerophospholipids in the fetal membranes, followed by prostaglandin biosynthesis and preterm labor.

Inhibitors of prostaglandin synthesis may delay par-

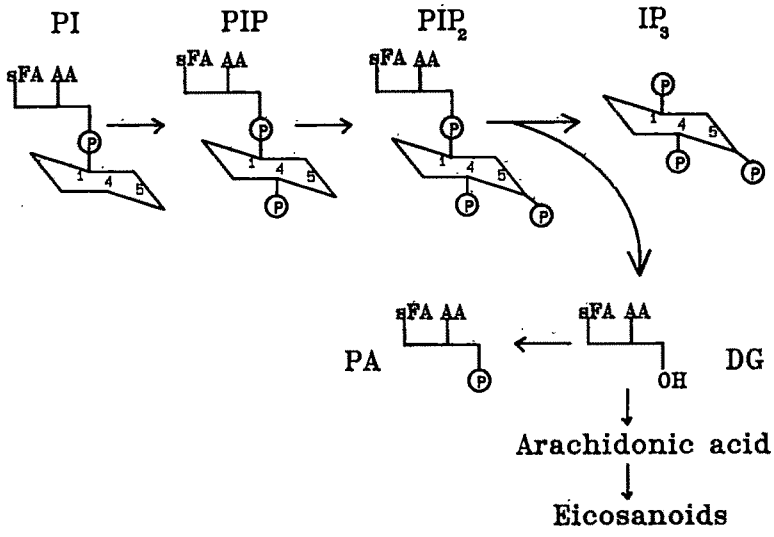


Fig. 3. Pathway of phosphatidylinositide metabolism. (*PI* = Phosphatidylinositol; *PIP* = phosphatidylinositol 4-phosphate; PIP_2 = phosphatidylinositol 4,5-bisphosphate; IP_3 = inositol trisphosphate; *sFA* = saturated fatty acid; *AA* = arachidonic acid; *PA* = phosphatidic acid; *DG* = diacylglycerol.) Eicosanoids comprise prostaglandins, thromboxane, and leukotrienes.

turition as shown in experimental animals.[98, 99] Aspirin and indomethacin are potent inhibitors of cyclooxygenase, which catalyzes the conversion of arachidonic acid to prostaglandins. In women, prolonged intake of large doses of aspirin can lengthen pregnancy,[100, 101] and administration of indomethacin can extend induction-abortion time in midtrimester abortion.[102] However, the use of these agents for treatment of preterm labor is disputed because they may cause premature closure of the ductus arteriosus, and aspirin, in particular, may lead to prolonged platelet dysfunction and bleeding.[103-106]

PGE_2, $PGF_{2\alpha}$ and some of their derivatives are useful tools in terminating pregnancy as in cases of an anencephalic or dead fetus[107, 108] where oxytocin is ineffective. They are less suited for the initiation of labor because of their nonspecificity. Thus they may contract other smooth muscles such as those of the blood vessels and intestinal tract. This may give rise to undesirable side effects, for example, nausea, vomiting, and diarrhea.

Overwhelming evidence in the literature suggests that there is interaction of prostaglandins and oxytocin and furthermore that the presence of prostaglandins is obligatory for a maximal response to oxytocin. Thus an increase in oxytocin sensitivity is prevented by administration of prostaglandin synthetase inhibitors.[109] In vivo, prostaglandins can lower the myometrial threshold to oxytocin.[110] In vitro, it has been shown that prostaglandins are needed for oxytocin action. Indomethacin, meclofenamate, and R-805, all potent inhibitors of prostaglandins synthetase (cyclooxygenase), antagonize the contractile effects of oxytocin but not of $PGF_{2\alpha}$ on the isolated uterus of the rat and humans.[111-116] Contraction of pregnant and nonpregnant human uteri, abolished by the addition of indomethacin, was reestablished in response to $PGF_{2\alpha}$ but not to oxytocin.[113]

In contrast to oxytocin, PGE and $PGF_{2\alpha}$ receptors change little in the human myometrium during pregnancy and labor. This would be in agreement with the small observed change in sensitivity to PGE through pregnancy and labor. Furthermore, there appears to be little change in prostaglandin receptors during the menstrual cycle. A single class of high-affinity binding sites was demonstrated for PGE_1 with an apparent equilibrium dissociation constant (K_D) of 3.65 nM for PGE_1 and 26.9 nM for $PGF_{2\alpha}$.[117]

In purified subcellular fractions from bovine and human pregnant myometrium, prostaglandin receptors were found in the cell membrane fraction and the sarcoplasmic reticulum, which is not surprising since prostaglandins are transported by the circulation and also originate inside the cell. The affinity of cell membrane and sarcoplasmic reticulum for PGE_2 was the same. The average K_D for PGE_2 and PGE_1 was 2.50 to 3.05 nM, with the same receptor specific for both these prostaglandins. $PGF_{2\alpha}$ and a series of inactive prostaglandins exhibited low competitive effects on PGE_2 binding.[118] The presence of the same receptor for PGE_1 and PGE_2 but of separate receptors for PGE and $PGF_{2\alpha}$ has been observed by many investigators.[117-120]

We may draw the conclusion that the physiologic changes that lead to human parturition are related to changes in the number of oxytocin receptors and to increased biosynthesis of prostaglandins.

Gap junctions. In addition, gap junctions appear in the myometrium of various animals during parturition and in humans during term and preterm labor. Gap junctions are areas of low resistance to the flow of cur-

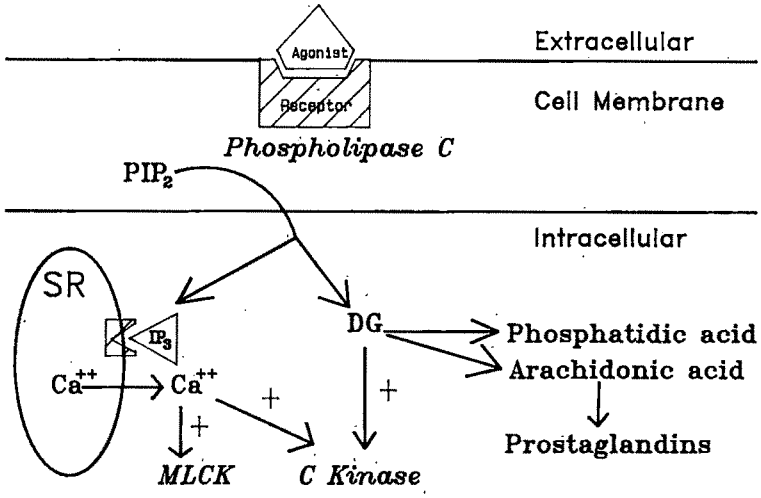


Fig. 4. Agonist-dependent second messengers: inositoltrisphosphate, calcium, and diacylglycerol. Agonist receptor-binding activates phospholipase C to hydrolyze phosphatidylinositol 4,5-bisphosphate *(PIP_2)* to inositol 1,4,5-trisphosphate *(IP_3)* and diacylglycerol *(DG)*. *MLCK* = Myosin light-chain kinase; *C Kinase* = protein kinase C. MLCK and C Kinase phosphorylate various proteins, leading to biologic responses.

rent. These cell-to-cell contacts serve to couple cells and allow synchronized muscle contractions in labor. Formation of gap junctions, at least in the rat and sheep, appears to be modulated by the interaction of various hormones, such as estrogen, progesterone, and prostaglandins.[121-123]

Second messengers

Cyclic AMP. The events leading from hormone binding to hormone action are mediated by so-called second messengers. Hormones bind to specific receptors at the outside of the cell membrane. The distribution and ligand specificity of the receptors determine the tissue response and production of a second messenger. The second messenger then transposes nonspecifically to the substrate inside the cell.

Adenosine-3′:5′-monophosphate (cyclic adenosine monophosphate, cAMP) has been identified as such a second messenger. Originally it was demonstrated that in the liver epinephrine releases cAMP, which in turn activates phosphorylase.[124] Based on this finding, the concept evolved that cAMP was the messenger for a multitude of other hormones. The action of cAMP is very well characterized as a messenger for epinephrine or isoproterenol, that is, for β-adrenergic agents. The uterine-relaxing action of β-adrenergic agents is well known. Indeed, some other relaxing agents have been shown to increase cAMP in the uterus, notably the anesthetic halothane.[125] Older theories of muscle contraction and relaxation, such as the Ying Yang theory, suggested that β-adrenergic agents brought about relaxation through the messenger cAMP and contractile agents work through cyclic guanosine monophosphate (cyclic GMP, cGMP).[126]

It was assumed that all relaxing agents work through cAMP and all contractile agents work through cGMP. Evidence has now accumulated to show that cAMP may not be an obligatory mediator of relaxation of the uterus. This hypothesis is based on the lack of correlation between the relaxing action of hormonal or pharmacologic agents and cAMP levels in the uterus or between tension and cAMP.[127, 128] Relaxation of the uterus produced by isoproterenol under various conditions in vitro does not always correlate with changes in membrane potential[129] or in tissue levels of cAMP.[130, 131] An explanation forwarded is that the increase in cAMP is localized and too small to be detected by chemical analysis of total tissue content of cAMP.[128] With whole-uterine muscle and a microsomal preparation from rat uterus, specific binding and cAMP production showed parallel dose-response curves as did relaxation.[132] However, it must be hypothesized that <10% of maximal cAMP production accounts for 50% of relaxation; this has not been proved.

Additional evidence for the lack of correlation of cAMP and relaxation comes from work on the role of prostaglandins in cAMP synthesis. In tissue slices PGE_1 and PGE_2, which contract the uterus, increased cAMP levels.[133] One might argue that results were obscured because of compartmentalization. However, in our laboratory, using uterine homogenates and partially purified subcellular fractions, PGE_2 and $PGF_{2\alpha}$, also elicited increased synthesis of cAMP, as did isoproterenol.[134] The claim that cAMP enhances calcium uptake in subcellular preparations[135, 136] was also untenable. With the use of purified microsomal preparations derived from the sarcoplasmic reticulum,[42] our laboratory showed that neither cAMP nor the protein kinase catalytic subunit increased calcium uptake.[134] Thus it appears to date that if cAMP has a relaxing action on the uterus, its mechanism is not in enhancing storage of calcium in intracellular organelles.

More recent evidence again involves cAMP in relaxation. cAMP may function primarily at the level of the actin-myosin interaction (see above and Fig. 1): in the phosphorylation of the enzyme myosin light-chain kinase by the cAMP–dependent protein kinase. This inhibits myosin light-chain kinase and thus myosin light-chain phosphorylation, thereby reducing actin-myosin interaction.

Cyclic GMP. Attempts to correlate cyclic guanosine monophosphate (cGMP) levels specifically with either contraction or relaxation have failed. Thus in carbachol-induced contractions, no change in cGMP was found in the rat uterus. In guinea pig myometrium, cGMP was increased, but in carefully timed experiments, contractions were shown to precede the rise in cGMP, and no changes in cAMP were observed.[137] Furthermore, no correlation between cGMP elevation and contraction or relaxation of rat myometrium could be established.[138]

Phosphoinositides. Since the nucleotide mechanism is inadequate to explain the link between hormone binding and contraction, what causes elevation of intracellular free calcium and contraction of the uterine smooth muscle cell? There is mounting evidence that binding of agonists to receptors, which leads to calcium mobilization, functions by hydrolyzing phosphoinositides.[139, 140] The phosphoinositides are minor components of cell membranes. Their structure is shown in Fig. 3. A phosphodiesterase (phospholipase C) hydrolyzes phosphatidylinositol 4,5-bisphosphate to diacylglycerol and D-myoinositol-1,4,5-trisphosphate (inositol trisphosphate). The inositol trisphosphate acts to mobilize intracellular calcium from the endoplasmic reticulum or sarcoplasmic reticulum, as it is called in smooth muscle (Fig. 4). Diacylglycerol activates protein kinase C, which phosphorylates various proteins.[139] The inositol trisphosphate is then broken down stepwise to inositol by phosphatases which are stimulated by diacylglycerol. In the cell membrane inositol is used for stepwise resynthesis of phosphatidylinositol. These reactions have been shown in many tissues.

Two experimental approaches are used to demonstrate these reactions. One is to demonstrate increased metabolites of phosphoinositides on receptor-agonist interaction; the other is to show an increase in free calcium in permeabilized cells or sarcoplasmic reticulum preparations on exposure to inositol trisphosphate. Increased hydrolysis of phosphoinositides and release of inositol trisphosphate have been observed in uterine strips on stimulation with oxytocin or carbachol.[141] In our laboratory, addition of inositol trisphosphate caused release of calcium from isolated sarcoplasmic reticulum preparations. This calcium release was concentration dependent, and at the highest concentration used, amounted to 40% of calcium releasable by an ionophore.[142] In permeabilized cells from the mesenteric artery, the amount of calcium released by inositol trisphosphate was sufficient to elicit a contraction.[143] Thus one is tempted to suggest that inositol trisphosphate may be the link between agonist-receptor binding, intracellular calcium rise, and uterine smooth muscle contraction.

Although there is strong evidence for the involvement of phosphoinositides in hormone-receptor binding and smooth muscle contraction, demonstration of specific inhibition of one of the enzymes involved in phosphoinositides breakdown (e.g., phospholipase C) is needed. Lack of contraction would establish the obligatory role of phosphoinositides. No specific inhibitors are available at this time.

The other product in the phosphodiesterase splitting of phosphoinositide is diacylglycerol. In addition to activating protein kinase C, diacylglycerol is converted to phosphatidic acid (an ionophore) or may release arachidonic acid (Fig. 3). The latter can give rise to prostaglandin and eicosanoid biosynthesis as shown in other tissues.[90, 144] Prostaglandins can cause release of calcium from isolated sarcoplasmic reticulum preparations of uterine smooth muscle and inhibit calcium uptake as shown in our laboratory[41, 42, 54, 55] and previously discussed. Thus their action may augment the calcium release initiated by inositol trisphosphate. On the other hand, prostaglandins may actually cause breakdown of phosphoinositides after specific binding to the cell membrane; no data are available on this. Presently we suggest that accelerated synthesis of prostaglandins, stimulated by hormone-receptor binding and increased phosphoinositide metabolism, may further increase intracellular free calcium and lead to uterine contraction and possibly labor.

Regulation of second messengers. Guanine nucleotides are known to be involved in modifying the cAMP system. This occurs through receptors communicating with a pair of guanine nucleotide-binding regulatory proteins (G proteins) in the cell membrane. One of these (G_s) enhances the activity of adenylate cyclase, the enzyme that catalyzes cAMP synthesis, while the other (G_i) inhibits.[145-147] Guanosine 5′-triphosphate (GTP)–liganded adenylate cyclase appears to be the active species, and hydrolysis of GTP to GDP is the primary mechanism of inactivation.[148] Thus GTP is an obligatory cofactor for hormonal stimulation of adenylate cyclase. In vitro some analogs of GTP can substitute for GTP, and in binding studies that use broken cell preparations a stable analog of GTP is desirable. Particulate preparations from the smooth muscle of the rat uterus were shown to specifically bind a GTP analog with a K_D of 1.0 μM.[149]

Although most information about G proteins relates to the adenylate cyclase system, G proteins also appear to be mediators in the inositol trisphosphate system. It is still uncertain what type of G protein is involved. Experiments in various tissues that use permeabilized cells (mast cells) or microsomal preparations (rat liver

or coronary artery) have shown that guanine nucleotide-binding proteins couple receptors with the hydrolysis of phosphoinositides.[150-152] Enhancement by GTP of inositol trisphosphate stimulated calcium release has been demonstrated in rat liver,[153] but not in vascular smooth muscle cells.[154] It has also been suggested that the guanine nucleotide regulatory mechanism may operate independently in mediating calcium release from intracellular stores in nerve cells.[155-157] To date no studies on the uterus have been reported.

Conclusions

Recent evidence has delineated the linking of receptor activation with the production of second messengers. Hormone receptor binding at the cell membrane initiates hydrolysis of phosphoinositides to inositol trisphosphate and diacylglycerol by phospholipase C. This reaction is possibly modified by an as yet unidentified GTP–binding protein. The inositol trisphosphate diffuses to the interior of the cell and releases calcium from the sarcoplasmic reticulum. Diacylglycerol can give rise to prostaglandins, which may release more calcium from the sarcoplasmic reticulum. In the presence of calmodulin, calcium then stimulates the myosin light-chain kinase to phosphorylate myosin; contraction follows. When the calcium level declines, the myosin light-chain kinase becomes inactive. The myosin is dephosphorylated by a phosphatase. Relaxation is furthered by phosphorylation of the myosin light-chain kinase by a cAMP–dependent protein kinase.

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CORRESPONDENCE

The detection of facial anomalies with ultrasound

To the Editors:

I feel that the article by Pilu et al. (Prenatal diagnosis of craniofacial malformations with ultrasonography. AM J OBSTET GYNECOL 1986;155:45-50) gives a falsely optimistic view of the sonographic prental diagnosis of craniofacial abnormalities, particularly those involving only the face.

Thirteen of the 14 patients in whom facial abnormalities were diagnosed were associated with major, readily diagnosable cranial malformations. In all of these cases, the cranial findings were of primary importance. Both of the patients in whom there were facial abnormalities not associated with obvious abnormal cranial findings (cases 15 and 16) were missed, although technically adequate examinations of the fetal face were obtained.

The sensitivity and specificity of sonographic examination in the diagnosis of fetal facial malformations remain to be demonstrated. The implication that its accuracy is currently well established is a disservice to both referring physicians and their patients, who may have unrealistic expectations. It also creates a dilemma for the consultant sonologist, who may be expected to deliver accuracy that may not be presently obtainable.

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Reply

To the Editors:

In reply to Dr. Koontz's letter, we feel that an important distinction has to be made. In our study (AM J OBSTET GYNECOL 1986;155:45-50), a selected obstetric population at high risk for fetal craniofacial malformation was considered. In each case a detailed and time-consuming ultrasound examination of the fetal face was performed. It is quite obvious that sensitivity and specificity of sonography would be much different in a low-risk obstetric population. With regard to this issue, the interested reader is referred to the very interesting article by Hegge et al.[1] that has been recently published.

Our experience indicates that a targeted examination of the fetal face yields great accuracy in the early prenatal diagnosis of many craniofacial anomalies whether the risk factor is a positive familial history, exposure to teratogens, or an associated fetal anomaly. However, a distinction has to be made. Hypotelorism, hypertelorism, cleft lip, and cleft lip–cleft palate are usually readily identifiable. Some craniofacial malformations, such as micrognathia, can probably be diagnosed only late in gestation, and it is likely that isolated cleft palate is not amenable to prenatal diagnosis. The considerable experience that was collected at Bologna University since our original manuscript was submitted for publication is well correlated with our preliminary data. Although a study with a design similar to ours has not yet appeared in the literature, the increasing number of recent reports dealing with both sophisticated investigation of subtle details of the normal anatomy of fetal facial structures[2] and rather large series of well-documented fetal craniofacial malformations diagnosed before birth[1,3] seem to support our view.

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Amniotic fluid trypsin and premature rupture of membranes

To the Editors:

We read the article, "The relationship between amniotic fluid trypsin activity and premature rupture of membranes," (AM J OBSTET GYNECOL 1986;155: 1043-8) by Kanayama et al. with interest and admiration. Nevertheless, we suggest that: (1) the principal conclusion is inaccurately expressed; (2) the case for amniotic fluid trypsin as the principal culprit in premature rupture of membranes remains unproved; and (3) other factors and information should be considered.

The authors write that they demonstrated trypsin activity in amniotic fluid. Rather, they have measured "trypsin-like" enzymatic activity, since the chromogenic substrate used (*N*-benzyl-L-isoleucyl-L-glutamyl-glycyl-L-argenine-p-nitroanilide) demonstrates proteolytic activity by a number of other serine proteases. Interestingly, this same substrate is used to assay coagulation factors II (prothrombin) and X (Stewart-Prower factor).[1,2] A nearly identical synthetic substrate is also used to measure plasminogen activator, plasmin, and urokinase activity.[3,4] Similarly, α_1-antitrypsin is not specific

Table I. Comparative measures of the tensile strength of human amniochorion after 24 hours at 37° C, 6% CO_2 incubation with various enzyme and control solutions; note that trypsin demonstrated the least impairment of membrane strength of enzymes measured

	N	*Mean %*	*SD %*
Buffer control	5	100	±12
Collagenase, 10 μg/ml	5	29	±19
Collagen, 20 μg/ml	5	11	±30
Trypsin, 10 μg/ml	5	92	±15
Trypsin, 20 μg/ml	5	86	±26
Elastase, 20 μg/ml	5	<10	
Pseudomonas aeruginosa (collagenase-producing)	5	<10	

and inhibits a number of trypsin-like proteases.[5] Therefore the effects of other serine proteases and antiproteases were likely also measured in these tests.

One of these enzymes measured was probably plasmin. Burgos et al.[6] and Jenkins et al.[7] demonstrated plasminogen/plasmin in amniochorion and suggested that focal concentrations of this protease participate in the pathogenesis of premature rupture of membranes.

It is likely that the 2-week gestational age difference between the premature rupture of membrane (31 weeks) and control (33 weeks) groups influenced the results. Pocknee and Abramovich[8] demonstrated that amniotic fluid trypsin (determined by radioimmunoassay) decreased rapidly near term. Milwidsky et al.[9] could find no differences in amniotic fluid or fetal membrane acid protease activity among women with or without premature rupture of membranes.

Other information suggest that trypsin is not as important in premature rupture of membranes as hypothesized: (1) Since trypsin-like activity is dispersed in amniotic fluid, it would act to "cover the waterfront" and diffusely weaken amniochorion. Studies performed during the past century do not demonstrate overall weakening of prematurely ruptured membranes.[10] (2) Furthermore, Lavery and Miller[11] noted that meconium exposure decreased strength and elasticity of fetal membranes. They suggested that intrauterine exposure to fetal gastrointestinal enzymes, prominently including trypsin present in meconium, predisposes to premature rupture of membranes. However, meconium staining and premature rupture of membranes could not be correlated in a large clinical review.[11] Previous studies consistently show localized inflammation and/or necrosis adjacent to the rupture site.[12, 13]

Membrane thinning reported here is much more dramatic than that measured by Artal et al.[10] In two earlier studies, Polishuk et al.[14, 15] noted great variability but no consistent evidence of fetal membrane thinning in premature rupture of membranes.

While examining the possibility that ascending, transcervical infection and/or inflammation may focally impair membrane integrity, we[16] have assessed the effects of various bacterial and mammalian proteases on the physical properties of fresh human amniochorion. Although bacterial collagenase (Sigma Chemical Co., St. Louis, Mo.), porcine elastase (Sigma), and collagenase-producing *Pseudomonas aeruginosa* markedly reduced measurements of fetal membrane tensile strength, bovine pancreatic trypsin preparations (Sigma) did not (Table I). Preliminary work shows that human protease–containing neutrophil lysate also reduces membrane strength and elasticity, whereas lymphocyte lysate and other control preparations do not. Sbarra et al.[17] induced similar membrane weakening with direct application of phospholipase A_2 to fetal membranes, which suggests that other enzymatic systems are also involved.

We suggest that highly localized fetal membrane impairment by bacterial-, maternal-, and/or fetal-derived proteases and other substances are probably more important in the pathogenesis of premature rupture of membranes than are trypsin-like substances dispersed in amniotic fluid. Certainly, focal destruction of protease inhibitors could allow damage by a number of proteolytic enzymes.

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Reply

To the Editors:

I thank Dr. James A. McGregor and his colleagues for their interest in this report. We also agree with their result that the main cause of premature rupture of membranes is latent chorioamnionitis. Premature rupture of membranes was caused by latent chorioamnionitis in about 60% of the cases in our study. However, chorioamnionitis is not recognized in all fetal membranes with premature rupture of membranes. For example, patients have no pathogenic bacteria or inflamed cells in or around the cervix, no uterine contractions, and their cervixs are closed. Furthermore, the microscopic observations of amniochorion do not contain chorioamnionitis. Even such cases sometimes produce premature rupture of the membranes. What is the mechanism of causation in such cases? We supposed that trypsin in amniotic fluid had proteolytic power according to the circumstances. Trypsin activity in such patients was higher than in controlled groups. We measured trypsin activity with S2222(KabiVitrum Co., Sweden), which is specific for trypsin. As shown in Table I, there is little possibility in measuring thrombin, factor Xa, and plasmin by S2222.

Moreover, we obtained data opposite to theirs, namely, that type III collagen in amnion was degraded by trypsin in amniotic fluid (bovine) and meconium in amniotic fluid (human) (Fig. 1). Thus under what circumstances does trypsin modify its proteolytic activity in amniotic fluid? We recently measured trypsin levels

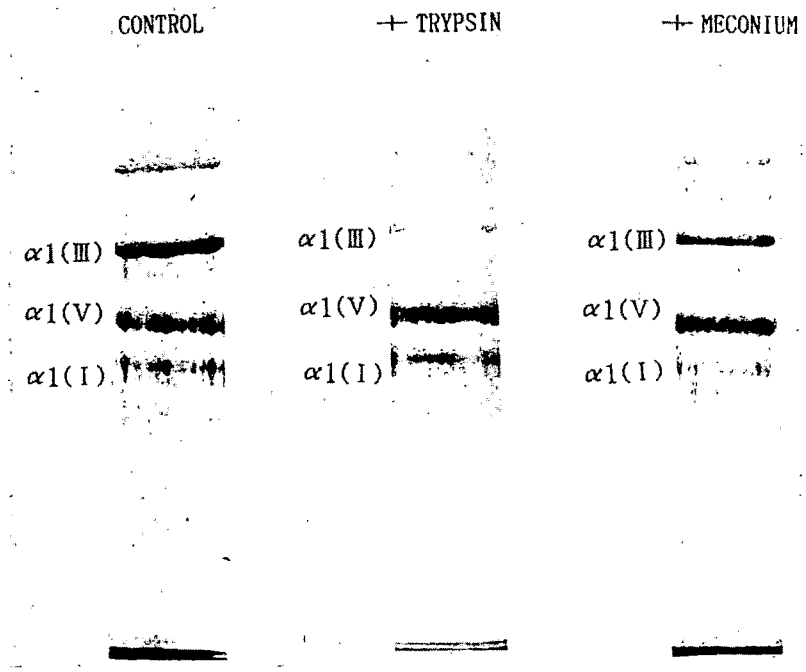


Fig. 1. Electrophoresis (7.5% polyacrylamide gel) of collagen in amnion. (*Left lane* = Normal amniotic collagen; *middle lane* = amniotic collagen (100 mg) treated with trypsin [10 mg] at 35° C for 6 hours; *right lane* = amniotic collagen [100 mg] treated with meconium [10 mg] at 35° C for 6 hours.) The migration of type I, III, and V α-chains is shown. Note the marked reduction of type III collagen in the middle lane and the slight reduction of that in the right lane.

Table I. The sensitivity of available substrates*

	Trypsin	*Thrombin*	*Factor Xa*	*Plasma kallikrein*	*Plasmin*	*Urokinase*
S2222	0.730	0.005	0.200	0.005	0.006	0.007

*By using 4×10^{-9} mol/L of enzyme, the following activities (Δ A/min) are obtained.

Table II. Trypsin concentration by radioimmunoassay in amniotic fluid

	PROM (N = 21)	*No PROM (N = 16)*	*Significance*
Trypsin (ng/ml)	42 ± 37	45 ± 46	NS

PROM = Premature rupture of membranes.

in amniotic fluid by radioimmunoassay (RIA-gnost Trypsin, Hoechst, West Germany). The results show that there is no difference between premature rupture of membranes and no premature rupture of membranes (Table II), which means that trypsin activity is controlled by the amount of α_1-antitrypsin and not by the amount of trypsin. Our article, which showed low levels of α_1-antitrypsin in cases of premature rupture of membranes, supports this theory.

α_1-Antitrypsin is a serine protease inhibitor that chiefly inhibits neutrophil elastase, chymotrypsin, and trypsin. α_1-Antitrypsin in amniotic fluid mainly comes from maternal blood. α_1-Antitrypsin is transferred from the uterine wall through the amniochorion to the amniotic cavity. Therefore a low value of α_1-antitrypsin in amniotic fluid in cases of premature rupture of membranes suggests that maternal serum has small amount of α_1-antitrypsin or that α_1-antitrypsin is consumed by protease in amniochorion. As a result, the α_1-antitrypsin that reaches the amniotic cavity in such cases does not completely neutralize the trypsin in the amniotic fluid. Secondarily activated trypsin may destroy amniochorion.

In summary, since α_1-antitrypsin is thought to be the main collagenolytic inhibitor in fetal membranes, a decrease of α_1-antitrypsin in fetal membranes helps clarify the cause of premature rupture of membranes.

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Possible vascular disruptive origin of hemifacial microsomia?

To the Editors:

In their report on chorionic villus sampling in 1000 cases, Hogge et al. (Chorionic villus sampling: Experience of the first 1000 cases. AM J OBSTET GYNECOL 1986;154:1249-56) mention four congenital anomalies in newborn infants from pregnancies monitored by chorionic villus sampling: Goldenhar syndrome, cloverleaf skull, achondroplasia, and polydactyly. The authors comment that "none of these anomalies appears to be related to the prenatal diagnostic procedure."

I do not quite agree with this statement with respect to the facioauriculovertebral (Goldenhar) complex. This malformation complex is not well defined and might include cases with different genetic and nongenetic etiology. Unfortunately, the authors did not present clinical details of their case. In animal experiments, Poswillo[1,2] induced congenital defects similar to those common in facioauriculovertebral complex by disrupting a fetal neck vessel. It is thought that a hematoma in the fetal neck damaged auricular and mandibular structures and thus finally led to the clinical picture of facioauriculovertebral complex. In a series of monozygotic twins discordant for congenital developmental defects, Schinzel et al.[3] reported an instance of facioauriculovertebral complex in the surviving twin of a fetus papyraceus and concluded that the defect in this instance might have been caused by embolization of thrombotic tissue or necrotic tissue from the deceased twin or its placental section through collaterals in a common placenta into a neck artery of the surviving twin.

In the case of the newborn with facioauriculovertebral complex, it was not mentioned from which area of the chorion the villi were obtained; however, it cannot be excluded that chorionic villus sampling caused a placental hematoma and that clotted blood or necrotic tissue had later embolized via the umbilical cord into a neck artery of the embryo or fetus. Thus facioauriculovertebral complex in a newborn from a pregnancy in which choriocentesis (or amniocentesis through the placenta) had been performed could well have a disruptive vascular origin. To obtain more insight into this possible pathogenesis, pregnancy histories of patients with facioauriculovertebral complex should be obtained with respect to any prenatal invasive diagnostic measures that were undertaken.

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Placental bed biopsy technique and vascular lesions

To the Editors:

I have read with interest the review by Robertson et al. (The placental bed biopsy: Review from three European centers. AM J OBSTET GYNECOL 1986;155: 401-12) about their extensive experience with the placental bed biopsy technique in three European centers during the past 30 years.

Vascular lesions of acute atherosis were found regularly in women with preeclampsia and idiopathic intrauterine growth retardation.[1-5] Similar vascular lesions were observed in women with chronic hypertension with or without superimposed preeclampsia,[6] in normotensive women with insulin-dependent diabetes,[6] and in endometrial spiral arteries from first-trimester abortions.[7]

The above mentioned lesions were described mainly in biopsy specimens from the placental bed but not from the parietal decidua. Interestingly, the authors said that "the best material to use to identify acute atherosis is the parietal decidua and not the placental bed or the basal plate of the placenta. The parietal decidua obviously is devoid of trophoblast invasion in spiral

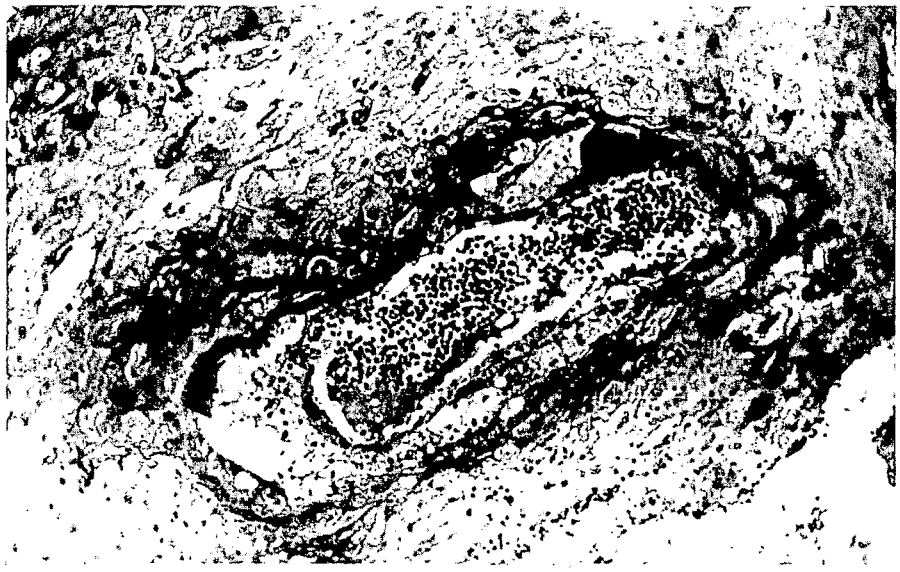


Fig. 1. Spiral artery of the basal plate with acute atherosis from a placenta from an idiopathic small for gestational age infant. (Hematoxylin-eosin stain; magnification ×100.)



Fig. 2. Dense deposits of IgM in two vessels with acute atherosis from a placenta from an infant with idiopathic intrauterine growth retardation. (Immunoperoxidase stain–hematoxylin counterstain; magnification ×100.)

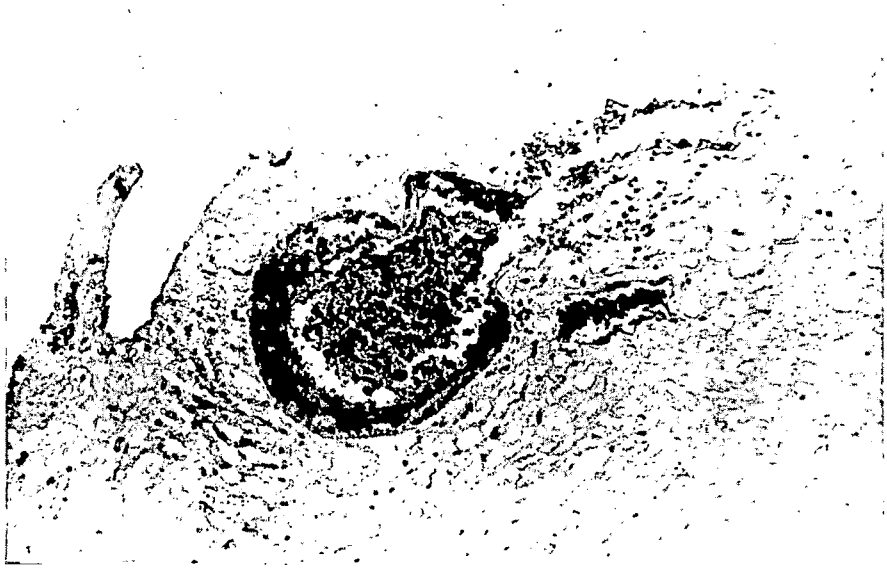


Fig. 3. Spiral artery of parietal decidua with acute atherosis from a placenta from a normotensive, intrauterine growth–retarded infant. Dense granular deposits of IgM are observed within the wall. (Immunoperoxidase stain–hematoxylin counterstain; magnification ×100.)

arteries. This vasculopathy is therefore seen in the parietal decidua in its 'pure' form, uncomplicated by preexisting trophoblast-induced vascular changes. Thus, in studies related mainly with vascular lesions, it is good practice to take biopsy specimens of parietal decidua in addition to those from the placental bed. Rolled-up strips of the placental membranes are a valuable additional source of parietal decidua containing portions of decidual spiral arteries, which may show acute atherosis.

The uteroplacental arteries present difficulties in interpretation with regard to pathologic lesions because their decidual segments have been altered by trophoblast invasion, and it becomes very difficult to differentiate the characteristic fibrinoid material in the vessel wall from the fibrinoid necrosis of an acute vasculopathy. Furthermore degenerated cells in the physiological changes, including trophoblast, accumulate lipid in their cytoplasm."

On the other hand, the authors state that there is little doubt that acute necrotizing lesions in small superficial vessels in the basal decidua but not in the myometrium or parietal decidua can be found in cases of intrauterine growth retardation unassociated with hypertension; their relationship with the acute atherosis characteristic of preeclampsia-eclampsia remains to be determined. However, we have found acute atherosis-like lesions predominantly in parietal decidua and also in the basal plate in placentas from patients with idiopathic intrauterine growth retardation[8, 9] (Fig. 1). These lesions were also seen in those placentas with associated preeclampsia[9] and also in patients with autoimmune diseases.[10]

Interestingly, heavy granular deposits of predominantly IgM (Figs. 2 and 3) and also C3 and Clq were found in lesions of acute atherosis but not in vessels with physiologic changes or an absence of trophoblastic invasion.[11] Furthermore, the frequent feature of leukocyte infiltration surrounding spiral arteries and endometrial glands in the placental bed has been shown to consist mainly of macrophages and T-lymphocytes, suggesting that these findings may be related to a local immune attack during pregnancy.[12] The same may be proposed for vascular lesions of acute atherosis.

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Reply

To the Editors:

Although we regard the comments made by Dr. Labarrere on our article as evidence that we have achieved our objective in stimulating discussion and interest in a neglected subject, we do not wish to take issue on the two main points he raises. Our article was intended only as a review of the technology and interpretation of the placental bed biopsy and not as a comprehensive assessment of the pathology of the pregnant uterus in gestational disorders. It may be that his assertion that vasculopathies in the decidua vera are a common feature of intrauterine growth retardation unassociated with hypertensive disorders will prove to be true. In our experience it has not been so, but in such cases we have concentrated our attention on the basal plate of the fetal placenta and the underlying placental bed. However, he is not correct in stating in his brief review of the literature that " the above mentioned lesions were described mainly in biopsies from the placental bed but not from the parietal decidua." Several of our earlier publications illustrate typical acute atherosis in the parietal decidua from cases of hypertensive disorders of pregnancy regardless of whether the cases were also associated with intrauterine growth retardation.

With regard to his proposal that uterine vasculopathies may be the result of an inappropriate immunologic reaction, we purposely did not dwell on this complex and contentious matter, since our review was essentially on morphologic aspects.

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Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia

To the Editors:

I was very interested to read the article "A comparison between laser excisional conization and laser vaporization for the treatment of cervical intraepithelial neoplasia" by Baggish (Am J Obstet Gynecol 1986;155:39-44), and I am prompted to make a few comments.

As a pathologist, I echo the sentiment that a serious shortcoming of laser vaporization is the lack of an adequate specimen to allow complete histologic evaluation of the disease. It is my belief that a not inconsiderable number of early invasive lesions are not being diagnosed and are being treated by vaporization.[1] The likelihood of missing a glandular abnormality in a punch biopsy specimen, particularly when cervical intraepithelial neoplasia is also present, is another reason to applaud the suggestion that excision be used as often as possible in place of vaporization.

Although this point of view is expressed in the introduction of the article, it is contradicted in the final paragraph where it is stated that "pure ectocervical lesions . . . may be effectively and easily eradicated by laser vaporization." The foregoing arguments recommend excision for all lesions whether or not the canal is involved.

If this final statement implies that all patients treated by laser excision in this series had lesions passing into the endocervical canal, then I am surprised that an excision 1.5 to 2.0 cm deep is considered adequate. Information from cone biopsies at the Samaritan Hospital, London, most of which were knife cones, shows that the lesion was completely excised in only 66% of those between 1.5 and 2.0 cm deep. I suggest that 2.0 cm is not a deep enough excision in those patients in whom the canal is involved.

I recommend that laser excision conization be considered for those patients whose lesion is purely ectocervical and in whom the canal is not involved. Furthermore, I question the necessity of a preceding biopsy; I believe that if the colposcopic examination is done by an experienced colposcopist who is satisfied that there is no canal extension, no colposcopic or cytologic suspicion of invasive disease, and no glandular abnormality, then it is safe to perform a laser excision conization at an outpatient visit. This saves an additional hospital visit for the patient and in a busy clinic serves to minimize the waiting list time for new patients. The excision in these patients without canal involvement need only be 1.0 to 1.5 cm deep. Since the whole lesion is being removed and subjected to histologic examination, an unsuspected invasive carcinoma or glandular abnormality would be diagnosed at that stage.

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REFERENCE

1. Anderson MC. Are we vapourizing microinvasive lesions? In: Sharp F, Jordan JA, eds. Proceedings of the fifteenth study group of the Royal College of Obstetricians and Gynaecologists. New York: Perinatology Press, 1986:127.

Reply

To the Editors:

In response to Dr. Anderson's letter about my article that compared laser excisional cone versus vaporization for the treatment of cervical intraepithelial neoplasia, I will give the following comments: (1) I am pleased that he agrees with the thesis that an excisional cone provides a more adequate specimen for sampling than does a punch biopsy and that by performing a laser excisional cone a greater margin of safety will be provided to those patients with endocervical extension. (2) If one has a "pure" ectocervical lesion, then this may be accurately and safely sampled via biopsies colposcopically directed and treated very adequately by laser vaporization to a depth of 7 mm. Worldwide statistics, including several publications from the United Kingdom,[1] consistently report long-term cure rates of more than 90%. (3) In contrast to a pathologist's viewpoint, as a gynecologist, I am concerned about deep cones that impinge on the internal cervical os and that may be a subsequent factor in infertility and pregnancy loss. A more sensible approach is to assess the depth of the cone by preoperative endocervical endoscopy or by immediate colposcopic examination of the excised and opened specimen. The latter is a technique that we have recently suggested and can demonstrate acetowhite epithelium but of course not abnormal vascular patterns at involved margins. (4) I cannot agree with Dr. Anderson that every patient with cervical intraepithelial neoplasia should have conization biopsy. Although my paper demonstrated that laser conization and vaporization equally share small risks, nevertheless, the cone procedure is more complex, two to three times deeper, and costlier. Similarly, I do not believe it is practical for cone biopsy to substitute for the office punch biopsy performed under colposcopic guidance.

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REFERENCE

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Conjoined twins in triplet pregnancy

To the Editors:

In reference to the interesting case report of Koontz et al. (Antenatal sonographic diagnosis of conjoined twins in a triplet pregnancy. AM J OBSTET GYNECOL 1985;153:230-1), we enclose a figure (Fig. 1) from April 1984 when we encountered a similar case.

In this case, diagnosis was made at 21 weeks' gestation.

The patient, a 36-year-old gravida 5, para 4, insisted on termination of the triplet pregnancy. Medical induction failed and hysterotomy was performed. Unfortunately, the placenta was found to be accreta and therefore hysterectomy was done.

In view of this, it would be more accurate to state that the case of Koontz et al. is the first to be reported and not "the first case . . . diagnosed antenatally with ultrasound."

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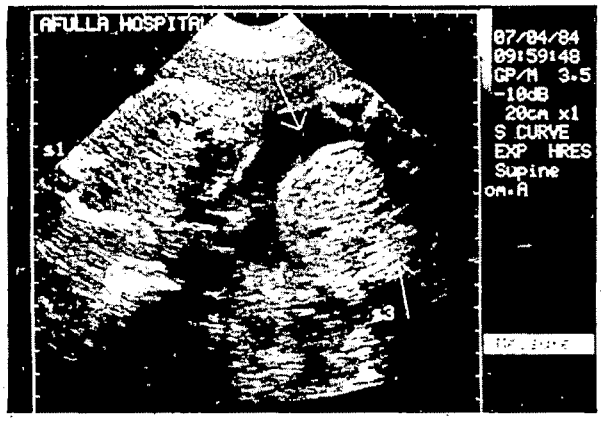


Fig. 1. Transverse section showing spines of the conjoined twins, S_1, S_2, and the single third fetus, S_3.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

The 3rd International Conference on Fetal and Neonatal Physiological Measurements, Malmö/Ronneby Brunn, Sweden, June 5-8, 1988. For additional information contact: The Secretariat, 3rd International Conference on Fetal and Neonatal Physiological Measurements, Department of Clinical Physiology, Lund University, Allmänna Sjukhuset, S-214 01 Malmö, Sweden. Tel.: +46 40 33 14 42.

Eleventh Annual Mid-Winter Symposium in Obstetrics and Gynecology, March 2, 3, and 4, 1988, Scottsdale Hilton Hotel, Scottsdale, Arizona. Co-Sponsored by the Phoenix OB/GYN Society and the Department of Obstetrics and Gynecology, Maricopa Medical Center. For information contact: The Secretary, Phoenix OB/GYN Society, 3412 N. Fourth Ave., Phoenix, AZ 85013. Tel.: (602) 241-1917.

The Ethical Battle Front and New Reproductive Technologies, Wednesday, December 9, 1987, The Engineering Society of Detroit. Sponsored by Hutzel Hospital/Wayne State University, Department of Obstetrics and Gynecology. Co-sponsored by the Michigan Section of the American College of Obstetricians and Gynecologists. For more information contact: Ms. Pat Dykowski, C. S. Mott Center, 275 East Hancock, Detroit, MI 48201. Tel.: (313) 577-1116.

Update in Colposcopy and Lower Genital Neoplasia and Update in Gynecologic Laser Surgery: Advances in Instrumentation and Techniques, The Breakers, Palm Beach, Florida, February 19 and 20, 1988. For further information contact: Dr. V. C. Wright, Department of Obstetrics and Gynecology, The University of Western Ontario, care of the Women's Clinic, 887 Richmond St., London, Ontario, Canada N6A 3J1. Tel.: (519) 438-1411.

The Agency for International Development (A.I.D.) has entered into a Cooperative Agreement with Jones Institute for Reproductive Medicine at the Eastern Virginia Medical School (EVMS). This agreement established CONRAD, a program of contraceptive research and development. A core of Jones Institute research scientists will conduct basic applied fertility regulation research at EVMS. Additionally, a large portion of the grant will be distributed to researchers at outside institutions such as universities, research centers, and private companies for the same purpose. As A.I.D. seeks new and improved contraceptive methods that are appropriate for developing country family planning programs, the CONRAD Program is interested in receiving proposals from researchers who can contribute to such technology. An area of particular interest is that of new or improved sterilization techniques, especially those that are nonsurgical and reversible in nature. Other areas include spermicides with viricidal activity and more acceptable and efficacious barrier methods. For more information or to request guidelines for proposals, please contact: Henry L. Gabelnick, Ph.D., Director Extramural Programs and Product Development, The CONRAD Program, 1611 N. Kent St., Suite 806, Arlington, VA 22209. Tel.: (703) 524-4744.

11th Annual Gold Coast OB/GYN Conference, "Current Clinical Concepts in Reproductive Endocrinology and Perinatology," February 8-10, 1988, Good Samaritan Hospital, West Palm Beach, Florida. For more information contact: Laura Lyons, CME Coordinator, Good Samaritan Hospital, P.O. Box 3166, West Palm Beach, FL 33402. Tel.: (305) 650-6236.

43rd Annual Postgraduate Assembly, OB-GYN Assembly of Southern California, February 11-14, 1988. Beverly Hilton Hotel, Beverly Hills, California. For information contact: Director of Medical Education, OB-GYN Assembly of So. California, 5820 Wilshire Blvd., #500, Los Angeles, CA 90036. Tel.: (213) 937-5514.

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INDEX TO ADVERTISERS

Albert Einstein Medical Center
Opportunity Available 56A
Ayerst Laboratories
Premarin 20A,21A,22A
Berlex Laboratories, Inc.
Levlen 25A,26A
Tri-Levlen 46A,47A,48A
Billings Clinic, The
Opportunity Available 67A
Carle Clinic
Opportunity Available 67A
Catherine McAuley Health Center
Opportunity Available 36A
Cedars-Sinai Medical Center
Opportunity Available 36A
CIBA Pharmaceutical Company
Estraderm 41A,42A,43A,44A
Davis Memorial Hospital
Opportunity Available 36A
FILMTEC, Inc.
Informed Consent Systems 56A
Flynn, Campbell, Collins, Executive Network
Opportunity Available 45A
Fort Collins Women's Clinic
Opportunity Available 61A
GeneralMed
Opportunity Available 45A
Kaiser Permanente, Kansas City
Opportunity Available 36A
Kaiser Permanente Medical Center
Opportunity Available 66A
Long Island College Hospital, The
Opportunity Available 61A
Lutheran General Hospital
Opportunity Available 56A
Mad River Community Hospital
Opportunity Available 61A
MacMillan Publishing
Books 53A
Maine Medical Center
Opportunity Available 63A
Marshfield Clinic
Opportunity Available 63A,64A,65A
Mary Imogene Bassett Hospital, The
Opportunity Available 63A
Marysville Medical Group
Opportunity Available 63A
Maxicare Health Plans, Inc.
Recruitment 37A
Mead Johnson Laboratories
Natalins Rx 14A
Medical Arts Clinic, S.C.
Opportunity Available 64A
Medical College of Wisconsin
Opportunity Available 66A
Medical Equipment Designs
HemoCue 8A,9A
Meeks, Rita
Opportunity Available 65A
Michael Reese Health Plan
Opportunity Available 65A
Mount Sinai Medical Center
Opportunity Available 62A
Multigon Industries, Inc.
Doppler 16A
Ortho Pharmaceutical Corporation
All-Flex 12A
Ortho-Novum 7/7/7 . Third Cover, Fourth Cover
Park Medical Center
Opportunity Available 45A
Pennsylvania Hospital
Opportunity Available 62A
Permanente Medical Group, Inc., The
Opportunity Available 62A,65A
Searle, G.D. & Company
Demulen 1/35 ..... 49A,50A,51A,52A
Sharplan Lasers
2100 ..... 6A
Smith Kline & French Laboratories
Cefizox 38A,39A,40A
Suny-Health Science Center
Fellowship 66A
Syntex Laboratories, Inc.
Femstat 57A,58A,59A,60A
Tri-Norinyl ..... Second Cover,1A,2A
Trover Clinic
Opportunity Available 66A
University of Calgary, The
Opportunity Available 61A
Upjohn Company, The
Provera 3A,4A
U.S. Air Force
Recruitment 54A,55A

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IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. Cases of hepatocellular carcinoma, although rare, have been reported in women taking oral contraceptives. Retrospective studies conducted in the United States and Britain report an increased risk of hepatocellular carcinoma in oral contraceptive users, particularly with increased duration of use. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **DRUG/LABORATORY TEST INTERACTIONS:** The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Revised 2/87

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Raritan, N.J. 08869-0602

ORTHO

Beyond low dose...

EXCELLENT RESULTS

Low incidence of nuisance side effects†
Low breakthrough bleeding
Low impact on selected metabolic parameters

21- and 28-day regimens

ORTHO-NOVUM 7/7/7 Tablets

TRADEMARK

norethindrone/ethinyl estradiol

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see brief summary of complete Prescribing Information on the preceding page.

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Raritan, New Jersey 08869-0602

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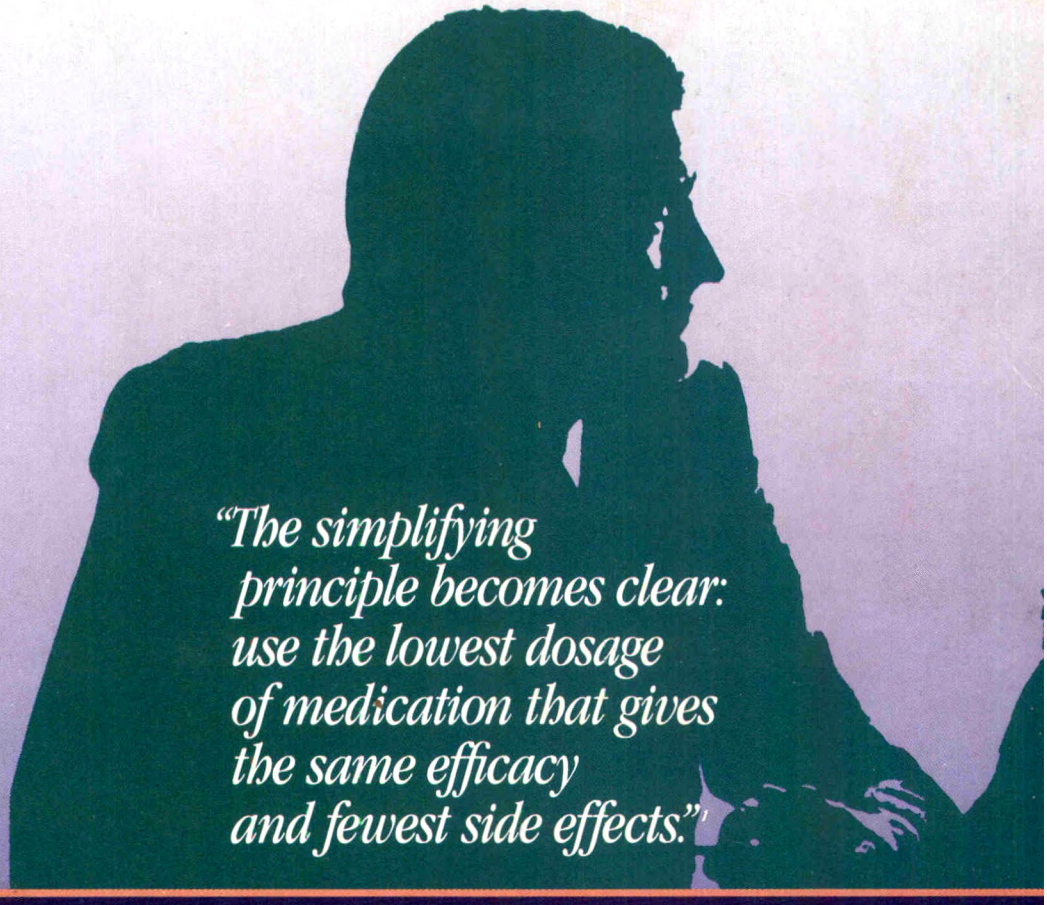
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Today's authoritie



"The simplifying principle becomes clear: use the lowest dosage of medication that gives the same efficacy and fewest side effects."[1]

Low Dose with Tri-Norinyl

- 29% less progestin per cycle than a 1/35 formulation[2*]

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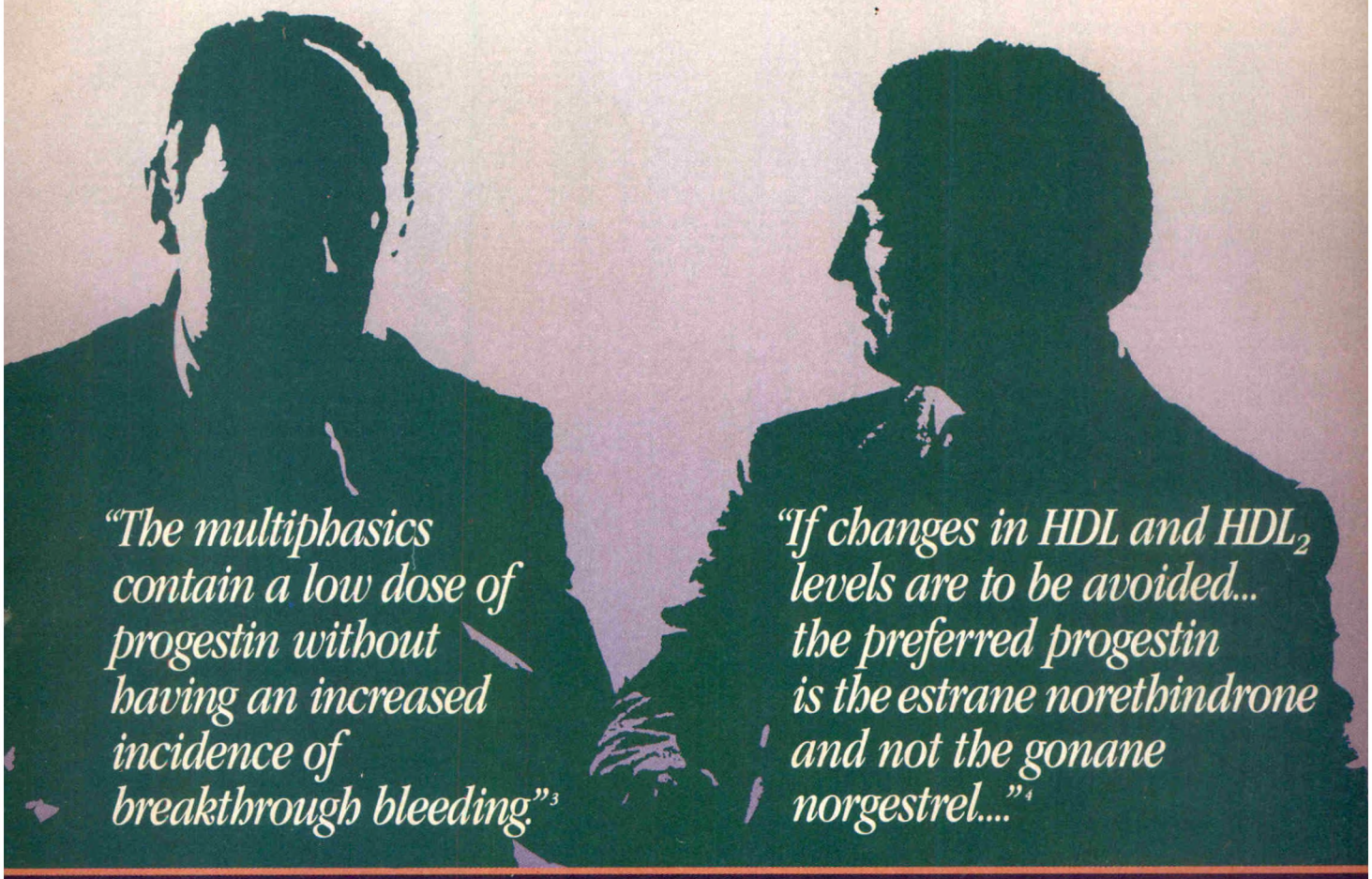
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2. Brett L: *Clinical Evaluation of Tri-Norinyl, a Triphasic Oral Contraceptive.* Palo Alto, CA, Syntex Laboratories, Inc., 1986.
3. Roundtable discussion. *Dialogues in Contraception* 1986;6:4.
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*1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Comparative data available from Syntex Laboratories, Inc.

No endorsement of Tri-Norinyl or any other Syntex oral contraceptive has been given by the authorities cited.

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on oral contraception:



"The multiphasics contain a low dose of progestin without having an increased incidence of breakthrough bleeding."[3]

"If changes in HDL and HDL_2 levels are to be avoided... the preferred progestin is the estrane norethindrone and not the gonane norgestrel...."[4]

Low BTB with Tri-Norinyl

- Extra midcycle protection that's unique among multiphasics
- Low BTB rates—equivalent to a fixed low-dose OC[2]

Low Impact with Tri-Norinyl

- Minimal lipid and cholesterol impact[4]
- Low incidence of nuisance side effects[2]

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

THE LOWEST-DOSE MULTIPHASIC OC WITH NORETHINDRONE

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of prescribing information on next page.

TRI - NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34 – 5/100,000; ages 35-44 – 33/100,000; ages 45-49 – 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 – 1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over – 1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS – RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. (See also Warnings, 4. *Liver Tumors.*) 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. Cases of hepatocellular carcinoma have been reported in long-term users of OCs.[105-107] An epidemiological study suggested a 4-fold increase in relative risk for hepatocellular carcinoma in OC users for 8 years or more, which increased to 7 in women with markers of hepatitis B. A second study suggested a risk of about 20 for use in excess of 8 years. 5. *Use in or immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones – estrogenic and progestational agents – during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero.* These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests that the incidence of *Chlamydia trachomatis* infection, the leading cause of nongonococcal PI.D., is increased by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Budd-Chiari syndrome, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chiblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure, premature supraventricular contractions. **Information for the Patient** (See Patient Package Insert).

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Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

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Contents

December
1987

CONSULTANTS

Consultants for the American Journal of Obstetrics and Gynecology 1325

PAPERS OF THE SOCIETY FOR GYNECOLOGIC INVESTIGATION

In vitro conversion of pregnenolone to progesterone in human term placenta and fetal membranes before and after onset of labor 1333
Firyal S. Khan-Dawood, Ph.D.
Chicago, Illinois

Metabolism of lipoproteins by human fetal hepatocytes 1338
Bruce R. Carr, M.D.
Dallas, Texas

The prevalence of autoantibodies in pregnant and nonpregnant women 1345
Phillip E. Patton, M.D., Carolyn B. Coulam, M.D., and Erik Bergstralh, M.S.
Rochester, Minnesota

(Contents continued on page 7A)

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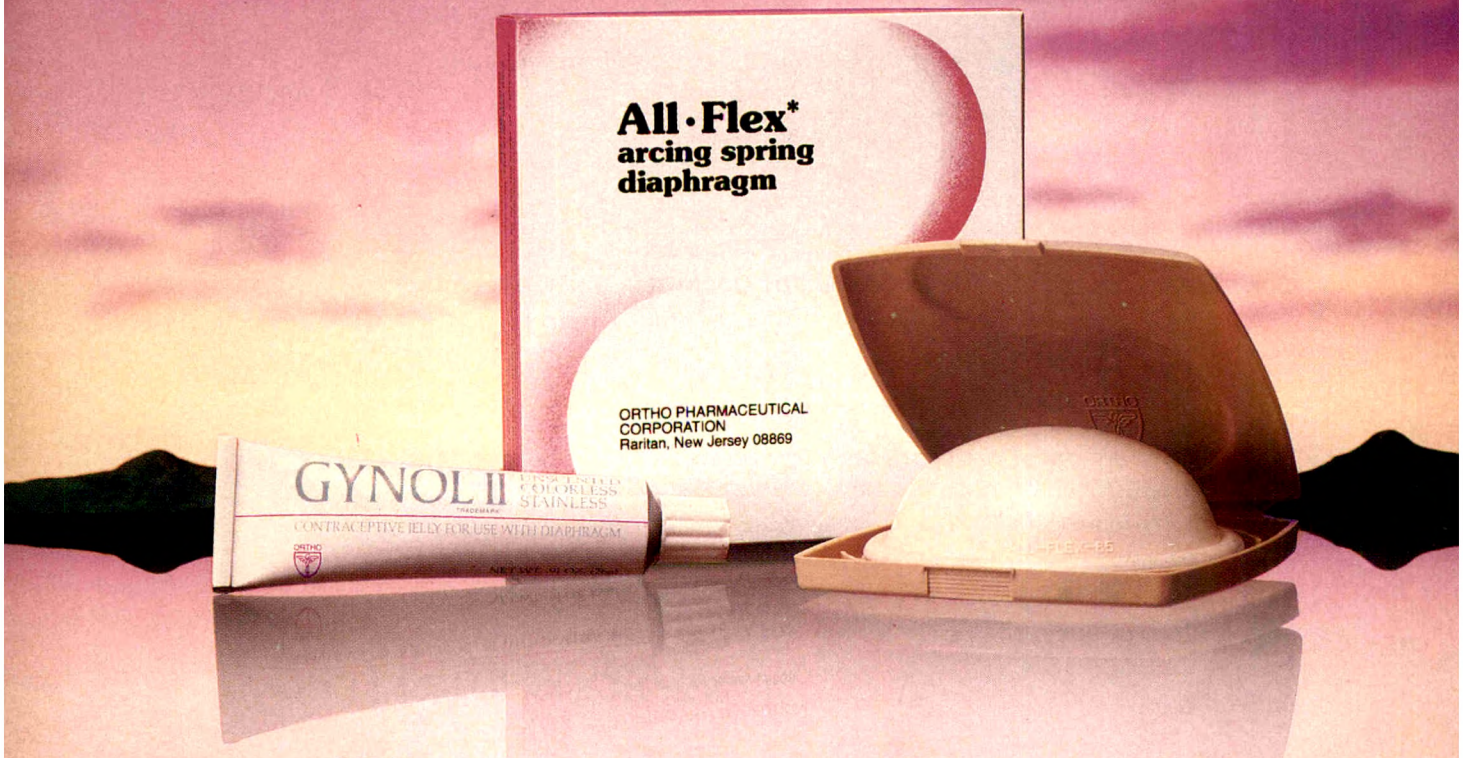
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Contents continued from page 5A

The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly 1351
Bruce R. Carr, M.D., and C. Richard Parker, Jr., Ph.D.
Dallas, Texas

Intrapartum vibratory acoustic stimulation of the human fetus during episodes of decreased heart rate variability 1355
Michael Y. Divon, M.D., Jeffrey J. Braverman, M.D., Denise A. Guidetti, M.D., Oded Langer, M.D., and Irwin R. Merkatz, M.D.
Bronx, New York

Postprandial walking exercise in pregnant insulin-dependent (type I) diabetic women: Reduction of plasma lipid levels but absence of a significant effect on glycemic control 1359
Dorothy Reycroft Hollingsworth, M.D., and Thomas R. Moore, M.D.
La Jolla, California

Restriction fragment length polymorphisms of the insulin gene hypervariable region in gestational onset diabetes mellitus 1364
Carole Ober, Ph.D., Candace J. Wason, R.N., M.S., Kelly Andrew, B.S., and Sharon Dooley, M.D.
Chicago, Illinois

Percutaneous umbilical transfusion in severe rhesus isoimmunization: Resolution of fetal hydrops 1369
Michael L. Socol, M.D., Scott N. MacGregor, D.O., Bruce W. Pielet, M.D., Ralph K. Tamura, M.D., and Rudy E. Sabbagha, M.D.
Chicago, Illinois

Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term 1375
Robert Gagnon, M.D., Cora Hunse, R.N., Lesley Carmichael, B.Sc., Fraser Fellows, M.D., and John Patrick, M.D.
London, Ontario, Canada

Fetal auditory brain stem response: Effect of increasing stimulus rate during functional auditory development 1382
Mark A. Plessinger, M.S., and James R. Woods, Jr., M.D.
Cincinnati, Ohio

Pituitary responses to synthetic corticotropin-releasing hormone: Absence of modulatory effects by estrogen and progestin 1387
J. H. Liu, M.D., D. D. Rasmussen, Ph.D., J. Rivier, Ph.D., W. Vale, Ph.D., and S. S. C. Yen, M.D., D.Sc.
La Jolla, California

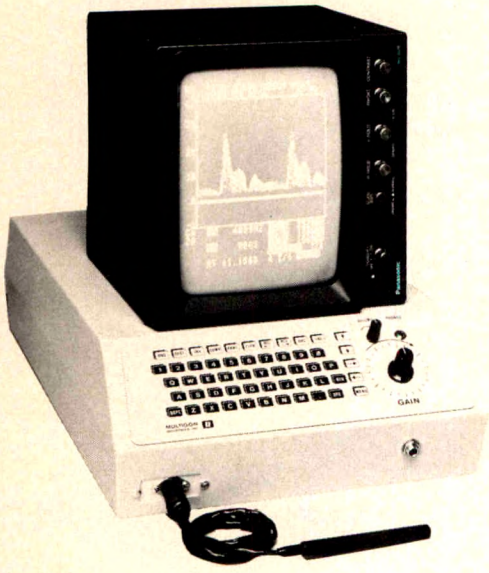
(Contents continued on page 9A)

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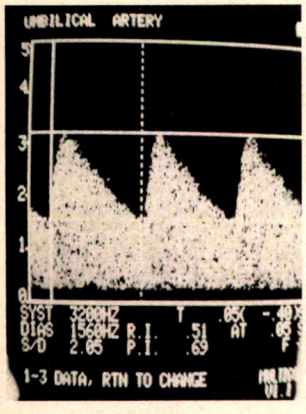


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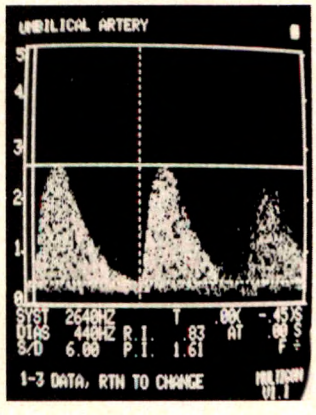
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Contents continued from page 7A

Effect of pregnancy on surgically induced endometriosis in cynomolgus monkeys 1392
Robert S. Schenken, M.D., Robert F. Williams, Ph.D., and Gary D. Hodgen, Ph.D.
San Antonio, Texas, and Norfolk, Virginia

Follicle-stimulating hormone is the main determinant of follicular recruitment and development in ovulation induction with human menopausal gonadotropin 1397
Michael Vermesh, M.D., and Oscar A. Kletzky, M.D.
Los Angeles, California

Human fetal breathing response to intravenous glucose is directly related to gestational age 1403
Margaret A. Harper, M.D., Paul J. Meis, M.D., James C. Rose, Ph.D., Melissa Swain, R.N., Julia Burns, B.S., and Bruce Kardon
Winston-Salem, North Carolina

Sonographic amniotic fluid measurement and fetal growth retardation: A reappraisal 1406
Robert M. Patterson, M.D., Thomas J. Prihoda, Ph.D., and Margeret R. Pouliot, R.N.
San Antonio, Texas

Ultrasonic prediction of birth weight in preterm fetuses: Which formula is best? 1411
Bruce W. Pielet, M.D., Rudy E. Sabbagha, M.D., Scott N. MacGregor, D.O., Ralph K. Tamura, M.D., and Seth L. Feigenbaum, M.D.
Chicago, Illinois

Effects of the antiprogesterone RU 486 in normal women. I. Single-dose administration in the midluteal phase 1415
Donna Shoupe, M.D., Daniel R. Mishell, Jr., M.D., Pekka Lahteenmaki, M.D., Oskari Heikinheimo, M.D., Lars Birgerson, M.D., Hosam Madkour, M.D., and Irving M. Spitz, M.D.
Los Angeles, California, New York, New York, Uppsala, Sweden, and Helsinki, Finland

Effects of the antiprogesterone RU 486 in normal women. II. Administration in the late follicular phase 1421
Donna Shoupe, M.D., Daniel R. Mishell, Jr., M.D., Mary Alice Page, Hosam Madkour, M.D., Irving M. Spitz, M.D., and Rogerio A. Lobo, M.D.
Los Angeles, California, and New York, New York

Serum CA-125 in the evaluation of benign adnexal cysts 1426
Donald E. Pittaway, M.D., Ph.D., Jamil A. Fayez, M.D., and James W. Douglas, M.D.
Winston-Salem, North Carolina

HLA antigen expression and induction by γ-interferon in cultured human trophoblasts 1429
Michael A. Feinman, M.D., Harvey J. Kliman, M.D., Ph.D., and Elliott K. Main, M.D.
Philadelphia, Pennsylvania, and Bronx, New York

(Contents continued on page 10A)

Contents continued from page 9A

Cerebrospinal fluid levels of magnesium in patients with preeclampsia after treatment with intravenous magnesium sulfate: A preliminary report 1435
Gary R. Thurnau, M.D., David B. Kemp, M.D., and Anthony Jarvis, M.B., B.Ch.
Oklahoma City, Oklahoma

Effects of cochlear ablation on local cerebral glucose utilization in fetal sheep 1438
Robert M. Abrams, Ph.D., Alastair A. Hutchison, M.B., Ch.B., Michael J. McTiernan, B.S., and Gerald E. Merwin, M.D.
Gainesville, Florida

Umbilical artery flow velocity waveforms and placental resistance: The effects of embolization of the umbilical circulation 1443
Brian J. Trudinger, M.D., Denise Stevens, B.Sc., Anita Connelly, B.Sc., J. Robert S. Hales, Ph.D., George Alexander, D.Sc., Laurence Bradley, B.Sc., Alan Fawcett, B.Sc., and Rosemary S. Thompson, Ph.D.
Westmead and Prospect, New South Wales, Australia

Continuous estrogen/progestin therapy in menopause 1449
Stanley G. Prough, M.D., Sezer Aksel, M.D., R. Herbert Wiebe, M.D., and Janet Shepherd, M.D.
Mobile, Alabama

Arachidonate lipoxygenase metabolites in amniotic fluid of women with intra-amniotic infection and preterm labor 1454
Roberto Romero, M.D., Ruben Quintero, M.D., Mohamed Emamian, M.D., Macor Wan, M.D., Camille Grzyboski, C.L.T., John C. Hobbins, M.D., and Murray D. Mitchell, D.Phil.
New Haven, Connecticut, and San Diego, California

Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor 1461
Roberto Romero, M.D., Mohamed Emamian, M.D., Macor Wan, M.D., Ruben Quintero, M.D., John C. Hobbins, M.D., and Murray D. Mitchell, D.Phil.
New Haven, Connecticut, and San Diego, California

Insulin resistance and abnormal ovarian responses to human chorionic gonadotropin in chronically anovulatory women 1468
James Kustin, M.D., Ralph R. Kazer, M.D., David I. Hoffman, M.D., Robert T. Chatterton, Jr., Ph.D., John N. Haan, M.S., Orville C. Green, M.D., and Robert W. Rebar, M.D.
Chicago, Illinois

Contents

Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua 1474
John R. G. Challis, Ph.D., D.Sc., and Michele Vaughan, B.Sc.
London, Ontario, Canada

Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: Fetal plasma concentrations and cardiorespiratory changes 1482
Charles A. Ducsay, Ph.D., Jeffry S. Thompson, B.S., Anne T. Wu, Ph.D., and Miles J. Novy, M.D.
Loma Linda and Palo Alto, California, and Beaverton and Portland, Oregon

Temporal changes in uterine activity and prostaglandin response to RU 486 in rhesus macaques in late gestation 1487
George J. Haluska, Ph.D., Frank Z. Stanczyk, Ph.D., Michael J. Cook, and Miles J. Novy, M.D.
Beaverton and Portland, Oregon

Cerebral oxidative metabolism in fetal sheep with multiple-dose ethanol infusion 1496
Bryan Richardson, M.D., John Patrick, M.D., Jacobus Homan, B.Sc., Lesley Carmichael, B.Sc., and James Brien, Ph.D.
London, Ontario, Canada

Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant 1503
Maurice L. Druzin, M.D., Michael Lockshin, M.D., Terri G. Edersheim, M.D., J. M. Hutson, M.D., A. L. Krauss, M.D., and Elizabeth Kogut, R.N.C.
New York, New York

Vaginal birth after cesarean 1510
Jeffrey P. Phelan, M.D., Steven L. Clark, M.D., Fidelia Diaz, M.A., and Richard H. Paul, M.D.
Los Angeles, California

The reliability and clinical use of a rapid phosphatidylglycerol assay in normal and diabetic pregnancies 1516
Samy A. Saad, M.D., Hossam E. Fadel, M.D., Kamal Fahmy, M.D., George H. Nelson, M.D., Ph.D., Mouhammed Moustafa, M.D., and Harry C. Davis
Augusta, Georgia, and Banha, Egypt

Fetal umbilical artery flow velocimetry in postdate pregnancies 1521
Denise A. Guidetti, M.D., Michael Y. Divon, M.D., Ralph L. Cavalieri, M.D., Oded Langer, M.D., and Irwin R. Merkatz, M.D.
Bronx, New York

(Contents continued on page 12A)

Contents continued from page 11A

Growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels compared in premature, small, average birth weight, and large infants 1524
Naguib A. Samaan, M.D., Ph.D., Pamela N. Schultz, R.N., B.S., Dennis A. Johnston, Ph.D., Robert W. Creasy, M.D., and Bernard Gonik, M.D.
Houston, Texas

Seminal plasma proteins of fertile and infertile men analyzed by two-dimensional electrophoresis 1528
Ramchandra R. Ayyagari, M.D., Asgerally T. Fazleabas, Ph.D., and M. Yusoff Dawood, M.D.
Chicago, Illinois

The effect of insulin on aromatase activity in isolated human endometrial glands and stroma 1534
John F. Randolph, Jr., M.D., Simon Kirpersztok, M.D., Jonathan W. T. Ayers, M.D., Rudi Ansbacher, M.D., M.S., Helle Peegel, M.S., and K. M. J. Menon, Ph.D.
Ann Arbor, Michigan

Uterine artery Doppler velocimetry: The significance of divergent systolic/diastolic ratios 1539
Harold Schulman, M.D., James Ducey, M.D., George Farmakides, M.D., Edwin Guzman, M.D., Denise Winter, R.N., B.S.N., Barbara Penny, R.N., and Chi-Lee, B.S.
Mineola, New York

The effects of membrane permeability and binding by human serum proteins on steroid influx into the rabbit uterus 1543
Kenneth A. Steingold, M.D., William M. Pardridge, M.D., Howard L. Judd, M.D., and Gautam Chaudhuri, M.D., Ph.D.
Los Angeles, California

The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation 1550
Molly E. Towell, M.B., Jorge Figueroa, M.D., Scott Markowitz, B.A., Beth Elias, B.Sc., and Peter Nathanielsz, M.D., Ph.D.
Ithaca, New York, and Hamilton, Ontario, Canada

Fetal heart rate response to vibratory acoustic stimulation predicts fetal pH in labor 1557
Terri G. Edersheim, M.D., J. Milton Hutson, M.D., Maurice L. Druzin, M.D., and Elizabeth A. Kogut, R.N.C.
New York, New York

Contents

BOOKS

Books received 1561

ITEMS

Items 1562

INDEX

Author index 1565

Subject index 1583

CORRECTIONS

Predictive value of early pregnancy glycohemoglobin in the insulin-treated diabetic patient (Key TC, Giuffrida R, Moore TR. 1987;156:1096-100) 1460

Inhibition of prolactin secretion by antiserum to the α- and β-subunits of gonadotropin (Blackwell RE, Garrison PN. 1987;156:863-8) 1520

Information for authors on pages 21A, 22A, and 23A

Index to advertisers on page 45A

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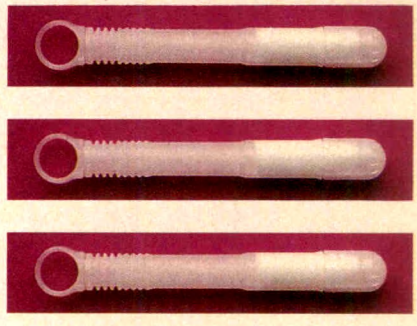
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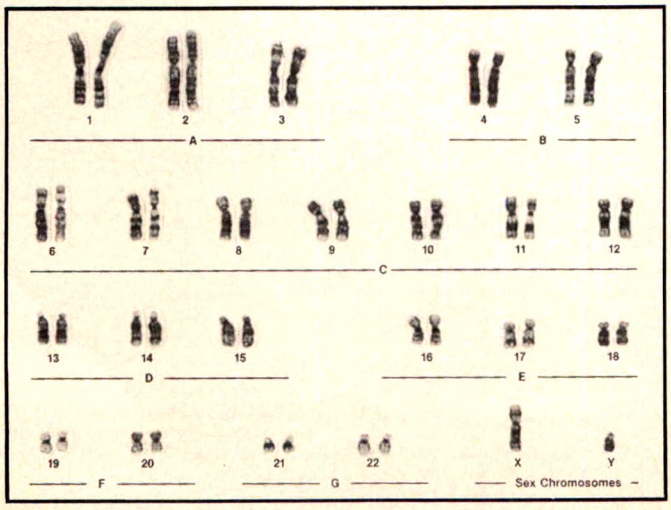
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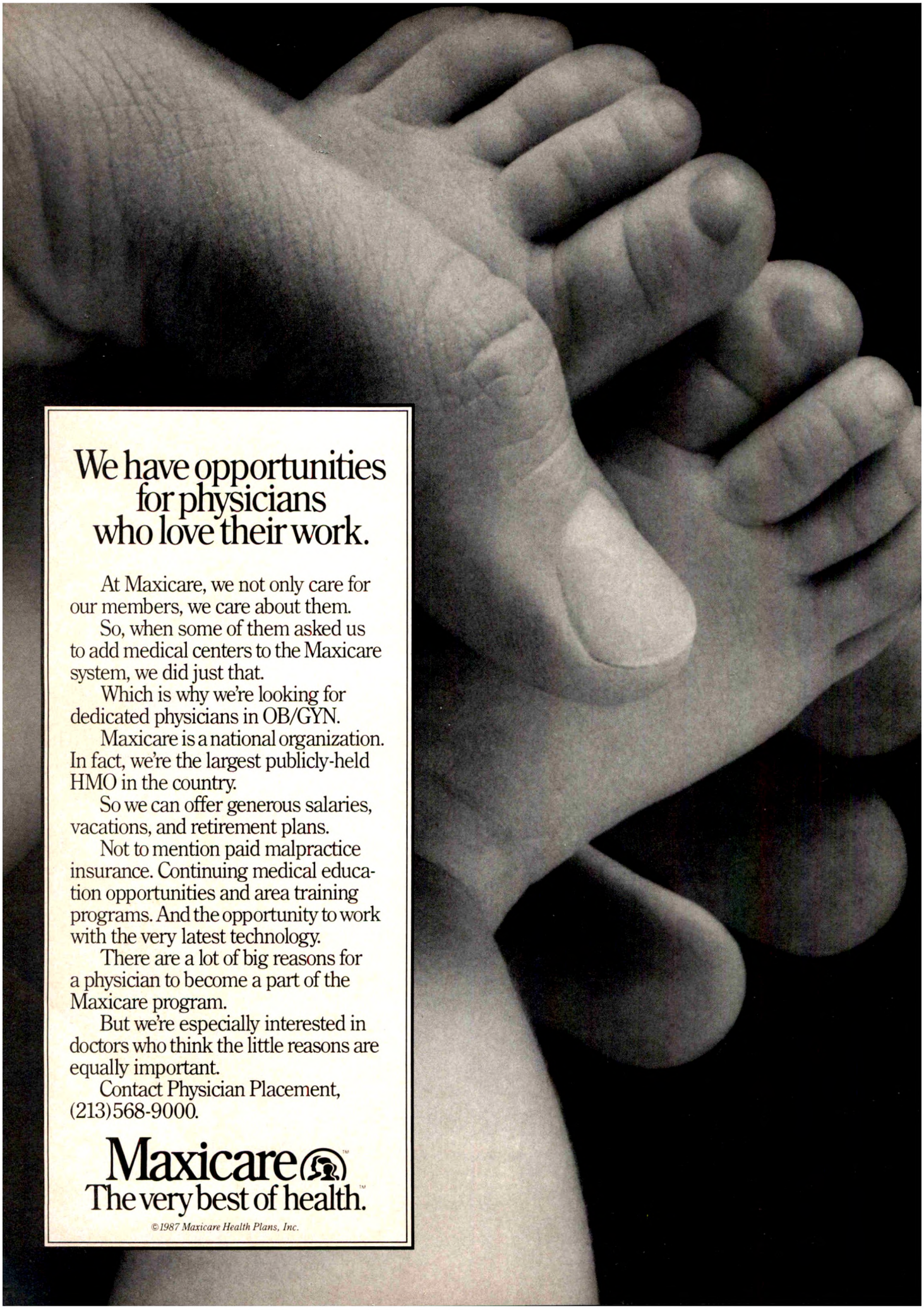
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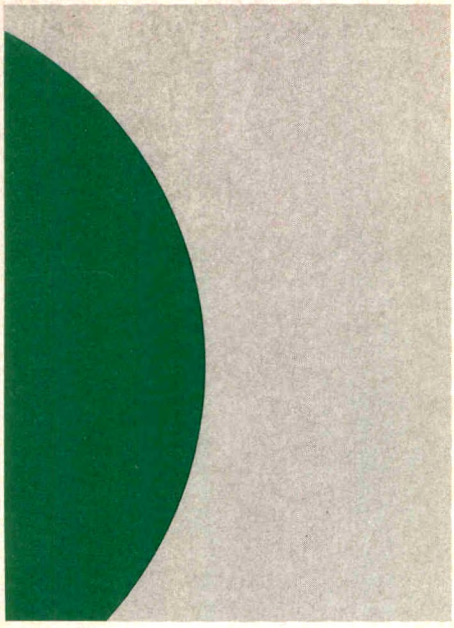
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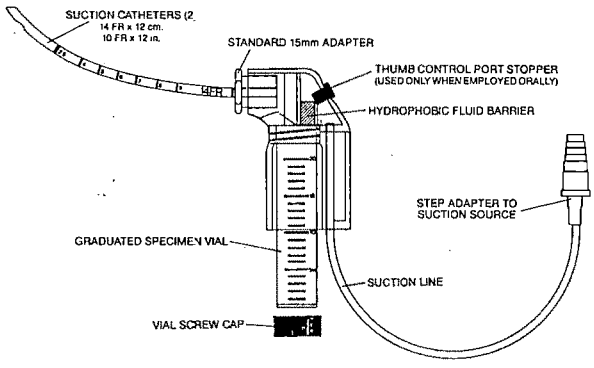
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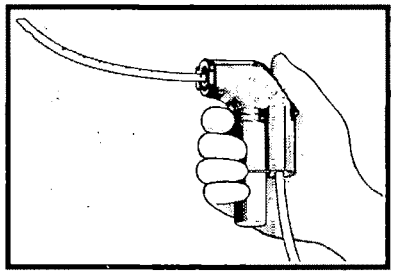
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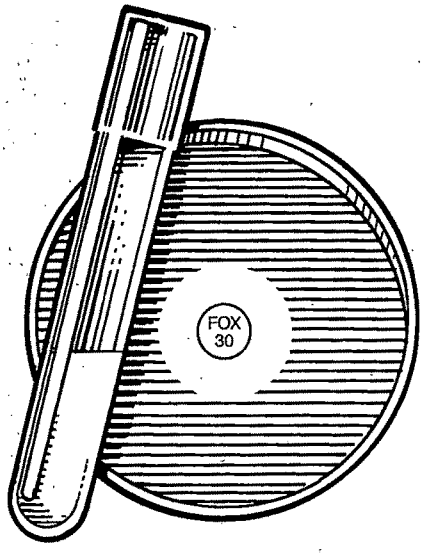
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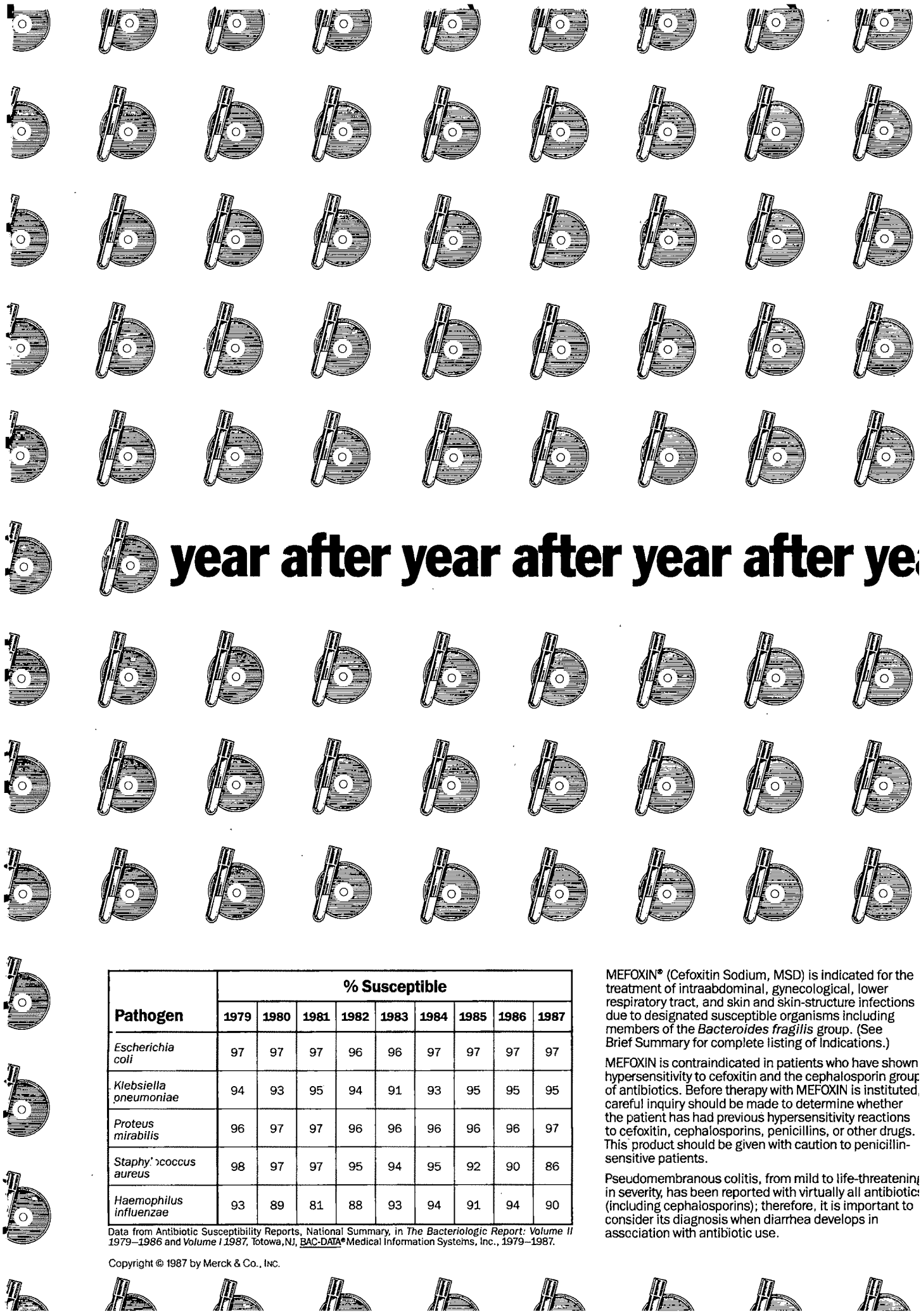
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Pathogen	% Susceptible								
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Escherichia coli	97	97	97	96	96	97	97	97	97
Klebsiella pneumoniae	94	93	95	94	91	93	95	95	95
Proteus mirabilis	96	97	97	96	96	96	96	96	97
Staphylococcus aureus	98	97	97	95	94	95	92	90	86
Haemophilus influenzae	93	89	81	88	93	94	91	94	90

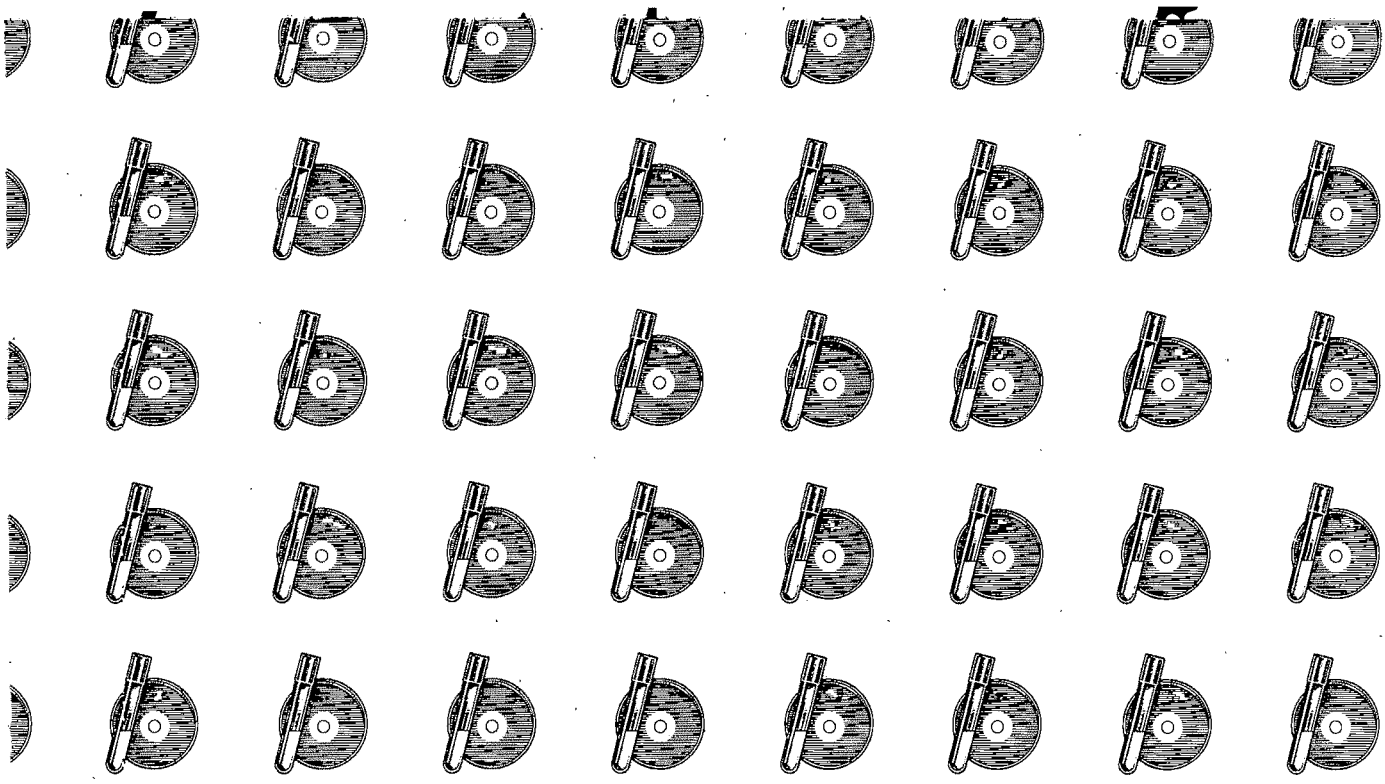
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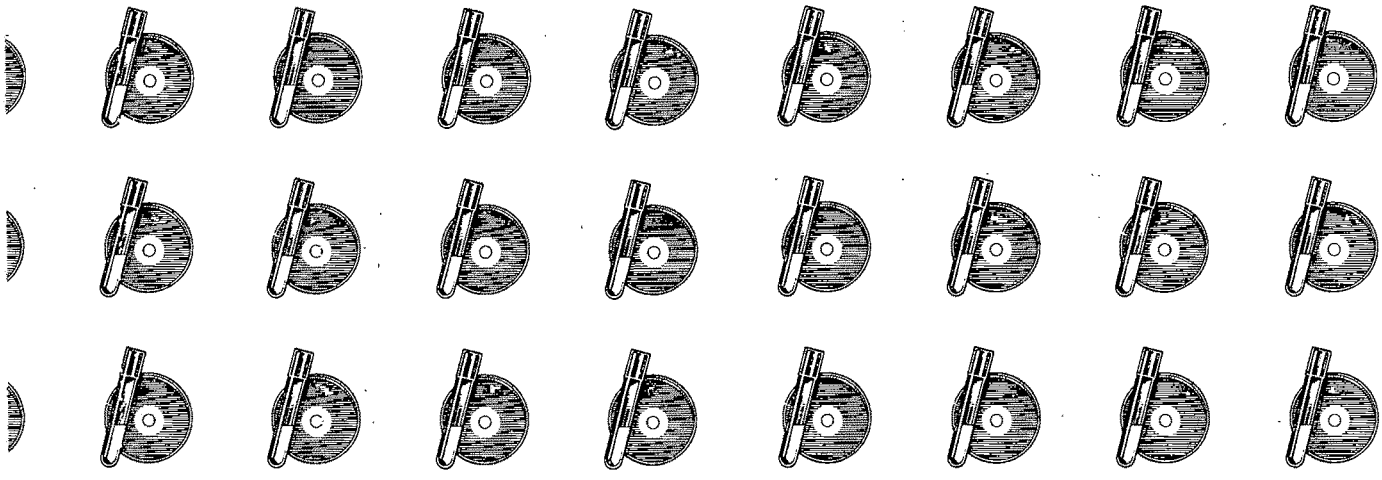
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Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST ‖ reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk: Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials, infusion bottles, and ADD-Vantage®# vials containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*

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DeCherney, Alan H., *New Haven, Connecticut*
DeVoe, Lawrence M., *Augusta, Georgia*
DeVore, Greggory R., *Salt Lake City, Utah*
Deigan, Eric A., *Newmarket, Ontario, Canada*
Depp, O. Richard, *Chicago, Illinois*
Dillon, Thomas, *Valhalla, New York*
Dilts, Preston V., *Ann Arbor, Michigan*
DiSaia, Philip J., *Irvine, California*
Dmowski, W. Paul, *Chicago, Illinois*
Dodds, William, *Columbus, Ohio*
Dooley, Sharon, *Chicago, Illinois*
Doran, Terence A., *Toronto, Ontario, Canada*
Dormer, Ken, *Oklahoma City, Oklahoma*
Droegemueller, William, *Chapel Hill, North Carolina*
Druzin, Maurice, *New York, New York*
Dudley, Denis K., *Ottawa, Ontario, Canada*
Duenhoelter, Johann H., *Puyallup, Washington*
Duff, W. Patrick, *Tacoma, Washington*
Dunn, Leo J., *Richmond, Virginia*

Dwane, Peter D., *Kingston, Ontario, Canada*
Effer, Sidney B., *Vancouver, British Columbia, Canada*
Eglinton, Gary, *Bethesda, Maryland*
Ehrlich, Clarence E., *Indianapolis, Indiana*
Elias, Sherman, *Memphis, Tennessee*
Elliott, John P., *Paradise Valley, Arizona*
Enkin, Murray W., *Hamilton, Ontario, Canada*
Entman, Steven, *Nashville, Tennessee*
Eschenbach, David A., *Seattle, Washington*
Eurard, J., *Providence, Rhode Island*
Evans, Arthur T., *Sacramento, California*
Evans, J. M., *Bristol, England*
Evans, Tommy N., *Denver, Colorado*
Evertson, Captain Larry, *Portsmouth, Virginia*
Fadel, Hossam, *Augusta, Georgia*
Fairweather, Denys, *London, England*
Fantl, Andrew, *Richmond, Virginia*
Farquharson, Duncan F., *Vancouver, British Columbia, Canada*
Faure, Nacia, *Quebec City, Quebec, Canada*
Ferenczy, Alex, *Montreal, Quebec, Canada*
Fishburne, John, *Oklahoma City, Oklahoma*
Fisher, Delbert, *Torrance, California*
Flowers, Charles, *Birmingham, Alabama*
Folkman, Judah, *Boston, Massachusetts*
Fragen, Robert J., *Chicago, Illinois*
Frederiksen, Marilynn C., *Chicago, Illinois*
Freedy, Lucy R., *Columbus, Ohio*
Freeman, Roger K., *Long Beach, California*
Friedman, Emanuel A., *Boston, Massachusetts*
Friedman, Chad, *Columbus, Ohio*
Friedrich, Eduard G., Jr., *Gainesville, Florida*
Frigoletto, Frederick D., *Boston, Massachusetts*
Friloux, Louis, *Orange, California*
Fu, Yaoshi, *Los Angeles, California*
Fuchs, Anna-Riita, *New York, New York*
Gabbe, Steven G., *Columbus, Ohio*
Gadek, James, *Columbus, Ohio*
Galask, Rudolph P., *Iowa City, Iowa*
Gall, Stanley, *Chicago, Illinois*
Gambrell, R. Don, Jr., *Augusta, Georgia*
Gant, Norman F., Jr., *Dallas, Texas*
Garbaciak, John A., Jr., *Cleveland, Ohio*
Gardner, Murray, *Sacramento, California*
Gare, Douglas J., *Toronto, Ontario, Canada*
Garfield, R. E., *Hamilton, Ontario, Canada*
Garite, Thomas, *Long Beach, California*
Garner, Peter R., *Ottawa, Ontario, Canada*
Gaziano, Emanuel P., *Minneapolis, Minnesota*
Gerbie, Melvin, *Chicago, Illinois*
Gerbie, Albert, *Chicago, Illinois*
Gerrity, M., *Glenview, Illinois*
Gibb, William, *Montreal, Quebec, Canada*
Gibbs, Ronald S., *San Antonio, Texas*
Gilbert, Raymond D., *Loma Linda, California*
Gilstrap, Larry C., III, *Dallas, Texas*
Gleicher, Norbert, *Chicago, Illinois*
Gluck, Louis, *Orange, California*
Goetzman, Boyd, *Davis, California*
Goland, Robin S., *New York, New York*
Golbus, Mitchell S., *San Francisco, California*
Golde, Steve, *Burbank, California*
Goldie, James H., *Vancouver, British Columbia, Canada*
Goldstein, Martin S., *New York, New York*
Goldstein, Donald, *Boston, Massachusetts*
Goodlin, Robert, *Denver, Colorado*
Goplerud, Clifford P., *Iowa City, Iowa*
Gordon, Alan, *Houston, Texas*
Gorwill, Robert H., *Kingston, Ontario, Canada*
Gown, Alan, *Seattle, Washington*
Grannum, Peter, *New Haven, Connecticut*
Gravett, Michael G., *Boise, Idaho*
Green, David, *Chicago, Illinois*
Greenspoon, Jeffrey S., *Los Angeles, California*
Greiss, Frank C., *Winston-Salem, North Carolina*
Grimes, David A., *Los Angeles, California*
Grimwood, Ronald E., *Columbus, Ohio*
Grix, Art, *Sacramento, California*
Grossman, John H., III, *Washington, D. C.*
Grumback, M. M., *San Francisco, California*
Guderian, A. Maynard, *Los Gatos, California*
Gupta, Sudhir, *Orange, California*
Gurpide, Erlio, *New York, New York*
Gusdon, John P., Jr., *Winston-Salem, North Carolina*
Hadlock, Frank, *Houston, Texas*
Haesslein, Hanns, *Sacramento, California*
Hale, Ralph W., *Honolulu, Hawaii*
Hallatt, Jack G., *Los Angeles, California*
Halme, Jouko, *Chapel Hill, North Carolina*
Halperin, Mary E., *Toronto, Ontario, Canada*
Hamilton, Emily, *Montreal, Quebec, Canada*
Hammond, Charles B., *Durham, North Carolina*
Hammond, Mary G., *Chapel Hill, North Carolina*
Handschmacher, Robert, *New Haven, Connecticut*
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Hansleigh, Paul, *Stanford, California*
Hanson, Frederick W., *Sacramento, California*
Harbert, Guy M., Jr., *Charlottesville, Virginia*
Harding, Paul R. G., *London, Ontario, Canada*
Harman, Christopher R., *Winnipeg, Manitoba, Canada*
Haseltine, Florence Pat, *New Haven, Connecticut*
Hatjis, Christos George, *Winston-Salem, North Carolina*
Hauth, John C., *Birmingham, Alabama*
Hayashi, T. Terry, *Pittsburgh, Pennsylvania*
Hayashi, Robert H., *Ann Arbor, Michigan*
Hazeltine, Florence, *Bethesda, Maryland*
Hemsell, David, *Dallas, Texas*
Henderson, Brian, *Los Angeles, California*
Henderson, Perry A., *Madison, Wisconsin*
Hendricks, Charles H., *Chapel Hill, North Carolina*
Hendricks, Susan K., *Seattle, Washington*
Hensleigh, Paul A., *San Jose, California*
Herbst, Arthur L., *Chicago, Illinois*
Hershey, Douglas W., *Denver, Colorado*
Hilgers, Robert, *Albuquerque, New Mexico*
Hill, Washington C., *Omaha, Nebraska*
Hill, Lyndon, *Pittsburgh, Pennsylvania*
Ho Yuen, Basil, *Vancouver, British Columbia, Canada*
Hobart, John M., *Evanston, Illinois*
Hobbins, John C., *New Haven, Connecticut*

Hobel, Calvin, *Los Angeles, California*
Hodgen, Gary, *Arlington, Virginia*
Hoffman, David I., *Chicago, Illinois*
Hollingsworth, Dorothy, *La Jolla, California*
Holly, Elizabeth A., *Belmont, California*
Holly, Roy, *Milwaukee, Wisconsin*
Hon, Edward, *Bradbury, California*
Horenstein, Janet M., *Los Angeles, California*
Horger, Edgar, *Charleston, South Carolina*
Horton, Richard, *Los Angeles, California*
Hosier, Don, *Columbus, Ohio*
Huddelston, John, *Jacksonville, Florida*
Hughey, Michael, *Evanston, Illinois*
Hulka, Jaroslav F., *Chapel Hill, North Carolina*
Hull, Walter, *Columbus, Ohio*
Hunter, Alasdair G. W., *Ottawa, Ontario, Canada*
Hurt, Glen, *Richmond, Virginia*
Huszar, Gabor B., *New Haven, Connecticut*
Iams, Jay D., *Columbus, Ohio*
Iffy, Leslie, *Newark, New Jersey*
Ingram, James M., *Tampa, Florida*
Israel, Robert, *Los Angeles, California*
Iwanicki, Stanislaw M., *Calgary, Alberta, Canada*
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James, Stanley, *New York City, New York*
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Jenkins, D. M., *Wilton, Cork, Ireland*
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Johnson, Timothy R. B., *Baltimore, Maryland*
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Jolly, Elaine E., *Ottawa, Ontario, Canada*
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Jones, Kenneth Lyons, *San Diego, California*
Jones, Richard O., *Aurora, Colorado*
Jones, Howard, *Norfolk, Virginia*
Jones, Howard W., III, *Nashville, Tennessee*
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Judd, Howard L., *Los Angeles, California*
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Kase, Nathan G., *New York, New York*
Katz, David, *Orange, California*
Katz, David S., *Davis, California*
Katz, Adrian, *Chicago, Illinois*
Kaufman, Raymond H., *Houston, Texas*
Kauppila, Antti, *Oulu, Finland*
Kaye, Helen, *Durham, North Carolina*
Keegan, Kirk A., *Orange, California*
Keifer, Walter S., *Seattle, Washington*
Keith, Louis, *Chicago, Illinois*
Kempers, Roger, *Rochester, Minnesota*
Kennedy, Thomas, *London, Ontario, Canada*
Kennedy, Melanie, *Columbus, Ohio*
Kent, Derecyk, *Orange, California*
Kerin, John F., *Los Angeles, California*
Killam, Allen, *Durham, North Carolina*
Kim, Moon, *Columbus, Ohio*
King, Howard J., *London, Ontario, Canada*
Kirk, Mary E., *London, Ontario, Canada*
Kirschbaum, Thomas, *Bethesda, Maryland*
Kirz, Donna S., *Chicago, Illinois*
Kitay, David, *Mobile, Alabama*
Kitzmiller, John L., *San Francisco, California*
Kleinman, Charles S., *New Haven, Connecticut*
Kletzky, Oscar A., *Los Angeles, California*
Klopper, Arnold, *Aberdeen, Scotland*
Knapp, R. C., *Boston, Massachusetts*
Knox, G. Eric, *Minneapolis, Minnesota*
Knuppel, Robert A., *Tampa, Florida*
Kochenour, Neil K., *Salt Lake City, Utah*
Kohorn, Ernest I., *New Haven, Connecticut*
Kopelman, Jerome N., *Honolulu, Hawaii*
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Kretchmer, Norman, *Berkeley, California*
Kretzschmar, Robert M., *Iowa City, Iowa*
Kubli, Frederick, *Heidelberg, Germany*
Kuhlman, Kathleen, *Chicago, Illinois*
Kuhnert, Betty, *Cleveland, Ohio*
Kurman, Robert John, *Washington, D. C.*
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Lacey, Conley G., *San Francisco, California*
Lamb, Emmet J., *Stanford, California*
Landon, Mark, *Philadelphia, Pennsylvania*
Lang, Roberto, *Chicago, Illinois*
Langmade, Charles F., *Pasadena, California*
Laragh, John, *New York, New York*
Lau, Rebecca Jane, *Salt Lake City, Utah*
Laube, Douglas William, *Iowa City, Iowa*
LeMaire, William J., *Miami, Florida*
Ledger, William J., *New York, New York*
Leier, Carl, *Columbus, Ohio*
Lenke, Roger R., *Toledo, Ohio*
Leppert, Phyllis, *New York, New York*
Leveno, Kenneth J., *Dallas, Texas*
Lewis, John, *New York, New York*
Lickrish, Gordon M., *Toronto, Ontario, Canada*
Liggins, G. C., *Auckland, New Zealand*
Lindheimer, Marshall D., *Chicago, Illinois*
Lipshitz, Jeffrey, *Calgary, Alberta, Canada*
Liston, Robert M., *Halifax, Nova Scotia, Canada*
Little, A. Brian, *Montreal, Quebec, Canada*
Liu, James H., *La Jolla, California*
Livingstone, Ronald A., *Winnipeg, Manitoba, Canada*
Lobo, Rogerio A., *Los Angeles, California*
Lomano, Jack, *Westerville, Ohio*
Longo, Lawrence D., *Loma Linda, California*
Low, James A., *Kingston, Ontario, Canada*
Lowe, Kenneth Gordon, *West Ferry, Dundee, Scotland*
Luck, Susan, *Chicago, Illinois*
Lurain, John R., *Chicago, Illinois*
Luther, Edwin R., *Halifax, Nova Scotia, Canada*
Lutz, Dennis J., *Minot, North Dakota*
Lye, Steven, *London, Ontario, Canada*

Lyons, Elliott R., *Toronto, Ontario, Canada*
MacDonald, Paul C., *Dallas, Texas*
MacKay, H. Trent, *Sacramento, California*
MacKenzie, Paul V., *Kingston, Ontario, Canada*
Macri, J. N., *Carle Place, New York*
Mahesh, Virendra, *Augusta, Georgia*
Main, Elliot, *Philadelphia, Pennsylvania*
Makowski, Edgar L., *Denver, Colorado*
Malkasian, George, *Rochester, Minnesota*
Mandel, Fred P., *Toronto, Ontario, Canada*
Manning, Frank A., *Winnipeg, Manitoba, Canada*
March, Charles, *Los Angeles, California*
Marchant, Douglas J., *Boston, Massachusetts*
Mariona, Federico G., *Detroit, Michigan*
Marquette, Gerald P., *Montreal, Quebec, Canada*
Marrs, Richard T., *Los Angeles, California*
Marshall, J. M., *Providence, Rhode Island*
Martin, James N., *Jackson, Mississippi*
Marx, G. F., *Bronx, New York*
Masterson, Byron J., *Louisville, Kentucky*
Mattison, Donald R., *Little Rock, Arkansas*
Mazzaferri, Ernest, *Columbus, Ohio*
McCarthy, Walter J., *Chicago, Illinois*
McDonald, John A., *Columbus, Ohio*
McDonough, Paul G., *Augusta, Georgia*
McGahan, John, *Sacramento, California*
McGanity, William, *Galveston, Texas*
McGrath, Michael M. J., *Kingston, Ontario, Canada*
McGregor, James A., *Denver, Colorado*
Medearis, Arnold L., *Los Angeles, California*
Meldrum, David, *Redondo Beach, California*
Menke, James, *Columbus, Ohio*
Merkatz, Irwin R., *Bronx, New York*
Merrill, James A., *Seattle, Washington*
Meschia, Giacomo, *Denver, Colorado*
Mestman, Jorge, *Los Angeles, California*
Metcalfe, James, *Portland, Oregon*
Meyer, Frank A., *Tel Aviv, Israel*
Milden, Randy S., *Cleveland, Ohio*
Milewich, Leon, *Dallas, Texas*
Millar, Nigel D., *Bristol, England*
Millard, Dietra A., *Chicago, Illinois*
Miller, Frank C., *Little Rock, Arkansas*
Miller, Richard K., *Rochester, New York*
Milligan, John E., *Toronto, Ontario, Canada*
Milunsky, Aubrey, *Boston, Massachusetts*
Minkoff, Howard, *Brooklyn, New York*
Mishell, Daniel R., Jr., *Los Angeles, California*
Mitchell, Bryan F., *London, Ontario, Canada*
Mitchell, Peter, *Edmonton, Alberta, Canada*
Mitchell, Murray, *San Diego, California*
Moawad, Atef H., *Chicago, Illinois*
Modanlou, Houchang D., *Long Beach, California*
Moghissi, Kamran, *Detroit, Michigan*
Mohide, Patrick T., *Hamilton, Ontario, Canada*
Molitch, Mark, *Chicago, Illinois*
Moore, Donald E., *Toronto, Ontario, Canada*
Morley, George, *Ann Arbor, Michigan*
Morris, H. H. B., *Oxford, England*
Morrison, John C., *Jackson, Mississippi*
Morrison, Ian, *Winnipeg, Manitoba, Canada*
Morriss, Frank H., Jr., *Iowa City, Iowa*
Morrow, C. Paul, *Los Angeles, California*
Mortel, Rodrigue, *Hershey, Pennsylvania*
Mueller-Heubach, Eberhard, *Pittsburgh, Pennsylvania*
Muggah, Henry F., *Ottawa, Ontario, Canada*
Mullen, Brendan J., *Toronto, Ontario, Canada*
Munsick, Robert A., *Indianapolis, Indiana*
Murata, Yuji, *Orange, California*
Murphy, Joan K., *Hamilton, Ontario, Canada*
Myerowitz, P. David, *Columbus, Ohio*
Myers, Stephen A., *Chicago, Illinois*
Myers, Ronald E., *Cincinnati, Ohio*
Naeye, Richard L., *Hershey, Pennsylvania*
Naftolin, Frederick, *New Haven, Connecticut*
Natale, Renato, *London, Ontario, Canada*
Nathanielsz, Peter W., *Ithaca, New York*
Neff, John, *Columbus, Ohio*
Nelson, Karin B., *Bethesda, Maryland*
Nelson, J. H., *Cincinnati, Ohio*
Nelson, Mike, *St. Louis, Missouri*
Nelson, George H., *Awendaw, South Carolina*
Nelson, Lewis H., III, *Winston-Salem, North Carolina*
Neutra, Raymond R., *Los Angeles, California*
Neuwirth, Robert, *New York, New York*
Newman, Roger B., *Charleston, South Carolina*
Newton, Michael, *Chicago, Illinois*
Newton, Niles, *Chicago, Illinois*
Nichols, David, *Providence, Rhode Island*
Nickel, James E., *Helena, Montana*
Niebyl, Jennifer, *Baltimore, Maryland*
Nimrod, Carl A., *Calgary, Alberta, Canada*
Nisker, Jeffrey A., *London, Ontario, Canada*
Niswander, Kenneth R., *Davis, California*
Noller, K. L., *Rochester, Minnesota*
Novy, Miles J., *Beaverton, Oregon*
O'Brien, Timothy, *Little Rock, Arkansas*
O'Connell, Gregory J., *Hamilton, Ontario, Canada*
O'Shaughnessy, Richard W., *Columbus, Ohio*
O'Toole, Robert, *Columbus, Ohio*
O'Dorissio, Tom, *Columbus, Ohio*
O'Leary, Jim, *Jacksonville, Florida*
O'Sullivan, Mary Jo, *Miami, Florida*
Oakes, Gary K., *Los Angeles, California*
Oh, William, *Providence, Rhode Island*
Oi, Richard H., *Sacramento, California*
Oki, Earle Y., *Sacramento, California*
Olive, David L., *Little Rock, Arkansas*
Olson, Ron, *Columbus, Ohio*
Ory, Steven J., *Rochester, Minnesota*
Ostergard, Donald R., *Long Beach, California*
Overstreet, James A., *Davis, California*
Owman, Christer, *Lund, Sweden*
Palmer, Judy, *Philadelphia, Pennsylvania*
Papageorgiou, Apostolos, *Montreal, Quebec, Canada*
Parer, Julian T., *San Francisco, California*
Parisi, Valerie M., *Houston, Texas*
Parker, Roy T., *Durham, North Carolina*
Pastorek, Joseph G., *New Orleans, Louisiana*

Patillo, Roland A., *Milwaukee, Wisconsin*
Patrick, John E., *London, Ontario, Canada*
Pauerstein, Carl J., *San Antonio, Texas*
Paul, Richard H., *Los Angeles, California*
Pearson, D. L. Clarke, *Chicago, Illinois*
Pearson, Jack, *Tucson, Arizona*
Peckham, Catherine S., *London, England*
Pepe, Gerald J., *Norfolk, Virginia*
Pergament, Eugene, *Chicago, Illinois*
Pernoll, Martin L., *New Orleans, Louisiana*
Perucca, E., *Pavia, Italy*
Peters, Ruth K., *Los Angeles, California*
Peterson, Bert, *Atlanta, Georgia*
Petra, Philip H., *Seattle, Washington*
Petrie, Roy H., *St. Louis, Missouri*
Phelan, Jeffrey P., *Los Angeles, California*
Phillipe, Mark, *Chicago, Illinois*
Phillips, Anthony F., *Farmington, Connecticut*
Pitkin, Roy M., *Los Angeles, California*
Piver, M. Steven, *Buffalo, New York*
Platt, Lawrence D., *Los Angeles, California*
Platzker, Arnold C., *Los Angeles, California*
Podratz, Karl C., *Rochester, Minnesota*
Pond, Donald G., *Toronto, Ontario, Canada*
Popkin, David R., *Saskatoon, Saskatchewan, Canada*
Porto, Manuel, *Orange, California*
Power, Gordon, *Loma Linda, California*
Prefontaine, Michel, *Ottawa, Ontario, Canada*
Pride, Sheila M., *Vancouver, British Columbia, Canada*
Prins, Robert, *Portland, Oregon*
Pritchard, Jack A., *Dallas, Texas*
Pupkin, Marcos J., *Baltimore, Maryland*
Queenan, John T., *Washington, D. C.*
Quilligan, Edward J., *Irvine, California*
Quinn, Patricia A., *Toronto, Ontario, Canada*
Quirk, J. Gerlad, *Little Rock, Arkansas*
Rankin, John H. G., *Madison, Wisconsin*
Ranney, Brooks, *Yankton, South Dakota*
Rao, V., *Louisville, Kentucky*
Ratnoff, Oscar, *Cleveland, Ohio*
Ravnikar, Veronica A., *Boston, Massachusetts*
Rayburn, William F., *Omaha, Nebraska*
Reagan, James, *Euclid, Ohio*
Rebar, Robert, *Chicago, Illinois*
Redman, Christopher, *Oxford, England*
Reed, Kathryn, *Tucson, Arizona*
Reid, Robert L., *Kingston, Ontario, Canada*
Reindollar, Richard, *Boston, Massachusetts*
Reiss, Rosemary E., *Columbus, Ohio*
Remington, Jack S., *Palo Alto, California*
Resnik, Robert, *Solana Beach, California*
Rettenmaier, Mark A., *Orange, California*
Richards, Douglas S., *Gainesville, Florida*
Richart, Ralph M., *New York, New York*
Riddick, Daniel, *Burlington, Vermont*
Ritchie, J. W. Knox, *Toronto, Ontario, Canada*
Roberts, Daniel K., *Wichita, Kansas*
Robertson, Jack, *Santa Maria, California*
Robertson, W. B., *Wimbledon, London, England*
Robertson, Andrew W., *Tacoma, Washington*
Roddick, J. W., *Springfield, Illinois*
Rodeck, C. H., *London, England*
Romero, Roberto, *New Haven, Connecticut*
Rose, James C., *Winston-Salem, North Carolina*
Rose, Peter, *Buffalo, New York*
Rosen, Mortimer, G., *New York, New York*
Rosenberg, Leon, *New Haven, Connecticut*
Rosenfeld, Charles R., *Dallas, Texas*
Rosenwaks, Zev, *Norfolk, Virginia*
Ross, Ian C., *Carlton, Australia*
Ross, Michael G., *Torrance, California*
Rossavick, I. K., *Houston, Texas*
Rowe, Timothy C., *Vancouver, British Columbia, Canada*
Rowe, R. D., *New Haven, Connecticut*
Roy, Michel, *Quebec City, Quebec, Canada*
Roy, Subir, *Los Angeles, California*
Rudolph, Abe, *San Francisco, California*
Rurak, Daniel W., *Vancouver, British Columbia, Canada*
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Ryan, Kenneth J., *Boston, Massachusetts*
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Sabbagha, Rudy E., *Chicago, Illinois*
Sachs, Larry A., *Columbus, Ohio*
Sahn, David, *San Diego, California*
Sakakini, Joseph, Jr., *El Paso, Texas*
Samuels, Philip, *Philadelphia, Pennsylvania*
Sandmire, Herbert F., *Green Bay, Wisconsin*
Sanfilippo, Joseph S., *Louisville, Kentucky*
Sarto, Gloria E., *Milwaukee, Wisconsin*
Sauerbrei, Eric E., *Kingston, Ontario, Canada*
Sawyer, Wilbur, *New York, New York*
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Schaefer, George, *La Mesa, California*
Schifrin, Barry, *Pasadena, California*
Schneider, Jack M., *Sacramento, California*
Schneider, Jan, *Philadelphia, Pennsylvania*
Schreiber, James Ralph, *Chicago, Illinois*
Schriock, Eldon, *Memphis, Tennessee*
Schulman, Harold, *Mineola, New York*
Schumacher, G. F. B., *Chicago, Illinois*
Schwane, James, *Tyler, Texas*
Schwartz, Peter E., *New Haven, Connecticut*
Schwarz, Richard, *Brooklyn, New York*
Sciarra, John, *Chicago, Illinois*
Scott, James R., *Salt Lake City, Utah*
Scully, Robert E., *Boston, Massachusetts*
Sealey, Jean, *New York, New York*
Seeds, John, *Chapel Hills, North Carolina*
Seibel, Machelle M., *Boston, Massachusetts*
Seitchik, Joseph, *San Antonio, Texas*
Sermer, Matthew, *Toronto, Ontario, Canada*
Settlage, Robert H., *Los Angeles, California*
Sever, John, *Bethesda, Maryland*
Seymour, Robert, *Montreal, Quebec, Canada*
Shapiro, Sander S., *Madison, Wisconsin*
Sheets, Ellen, *Orange, California*
Shenker, Lewis, *Tucson, Arizona*
Shennan, Andrew T., *Toronto, Ontario, Canada*
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Sherwood, O. David, *Urbana, Illinois*

Shime, Jerry, *Toronto, Ontario, Canada*
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Shoupe, Donna, *Los Angeles, California*
Shulman, Sidney, *New York, New York*
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Silva, Joseph, *Sacramento, California*
Silver, Richard K., *San Antonio, Texas*
Simmons, Michael, *Salt Lake City, Utah*
Simpson, Joe Leigh, *Memphis, Tennessee*
Simpson, Charles W., *Saskatoon, Saskatchewan, Canada*
Simpson, Evan R., *Dallas, Texas*
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Socol, Michael, *Chicago, Illinois*
Soderstrom, Richard M., *Seattle, Washington*
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Solomon, Samuel, *Montreal, Quebec, Canada*
Sonek, Mojmir, *Newark, Ohio*
Sotos, Juan, *Columbus, Ohio*
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Stallard, Richard, *Columbus, Ohio*
Stanczyk, Frank Z., *Los Angeles, California*
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In vitro conversion of pregnenolone to progesterone in human term placenta and fetal membranes before and after onset of labor

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To determine the role of placental progesterone in the onset of labor, tissue progesterone concentrations and in vitro conversion of tritiated pregnenolone to progesterone were examined in the placentas, chorion, and amnion of 12 term pregnancies delivered by cesarean section with or without labor and vaginal delivery. In all instances, the placenta had significantly higher progesterone concentrations than the chorion or amnion. Progesterone concentrations in the chorion were significantly lower after the onset of labor than before. The placenta and chorion but not the amnion converted pregnenolone to progesterone; the placenta had a significantly greater conversion rate than the chorion in all instances. Placental conversion of pregnenolone to progesterone increased significantly after labor to reach a maximum with vaginal delivery. Chorionic conversion of pregnenolone to progesterone was significantly increased after vaginal delivery but was similar after cesarean section with or without labor. The reduced tissue progesterone in the chorion, together with enhanced placental and chorionic production of progesterone from its precursor, pregnenolone, suggest that rapid metabolism and increased binding of progesterone locally in the placenta and membranes may lead to the local withdrawal or decline of progesterone and onset of labor. (AM J OBSTET GYNECOL 1987;157:1333-7.)

Key words: Progesterone, pregnenolone, placenta, fetal membranes, labor

Although a decline in circulating progesterone has been found in some species at the approach of the onset of labor,[1-3] such a clear change has yet to be convincingly demonstrated in human labor. Because the placenta is the major source of progesterone in late human pregnancy, progesterone production and local concentrations in the placenta and membranes and their direct contact with the uterus may be more relevant to the onset of uterine activity in women. Thus a change toward either reduced placental production or increased placental metabolism of progesterone at the approach of or during labor could provide for a local withdrawal of progesterone with accompanying reduction of the threshold of uterine muscle excitability. Since such local changes in placental progesterone metabolism could potentially modulate uterine activity, studies of the placental progesterone-synthesizing reserve capacity might provide useful information toward a better understanding of the contribution of progesterone in the onset of labor.

One approach would be to examine the in vitro bioconversion of pregnenolone to progesterone, which reflects the enzyme complex 5-ene-3B-hydroxysteroid dehydrogenase and 5-ene-4-ene isomerase but specifically the placental steroidogenic enzyme 5-ene-3B-hydroxysteroid dehydrogenase. Therefore this study was carried out to determine the tissue concentrations of progesterone and the conversion rates of pregnenolone to progesterone in placental and fetal membrane tissues before and after the onset of human labor and parturition.

Material and methods

Patients. Placentas and membranes were obtained from 12 women with uncomplicated term (38 to 40 weeks) pregnancy. Six women had spontaneous normal labor and vaginal delivery, three had spontaneous labor but underwent cesarean section for breech or other malpresentation, large fetus, or previous cesarean section after they were in established first stage of labor, and three had elective cesarean section.

Tissue collection and preparation. Twelve placentas with membranes attached were collected immediately at the time of delivery. Each placenta was immediately

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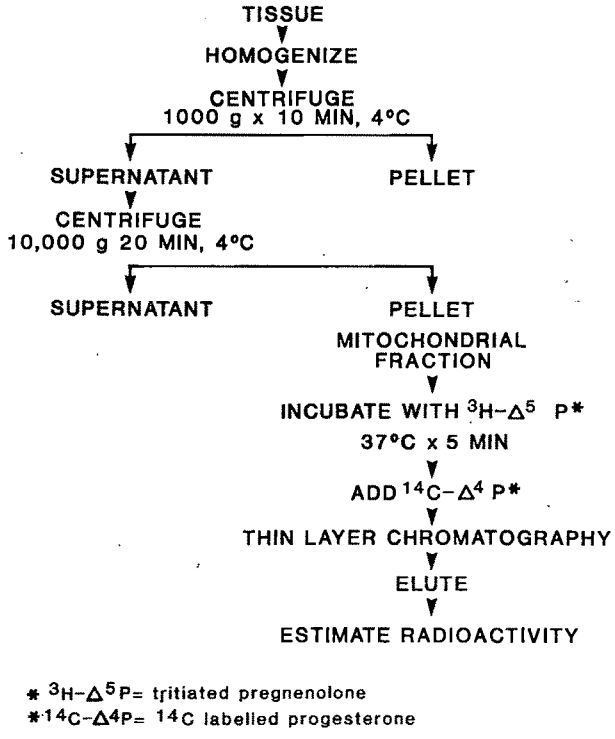


Fig. 1. Schematic outline of the steps for obtaining mitochondrial preparation from placental tissue, chorion, or amnion for use in determining the in vitro bioconversion of pregnenolone to progesterone.

placed in crushed ice in the operating room and rapidly transported to the laboratory. All manipulations were carried out at 4° C. Each placenta was dissected and samples (2 gm each) of cotyledons, chorion, and amnion were removed separately and washed in ice-cold 0.9% sodium chloride solution to remove blood. Each washed tissue specimen was either homogenized immediately or dried over filter papers and kept frozen at −70° C until ready for analysis. The method for homogenizing the tissue and obtaining mitochondrial preparation is outlined in Fig. 1. Samples of the final tissue preparation were verified by electron microscopy to be mitochondria. The cytosol fraction was used for determining intracellular concentration of progesterone.

Pregnenolone to progesterone conversion. In vitro bioconversion of pregnenolone to progesterone by placental cotyledon, amnion, and chorion was determined with the mitochondrial preparation of the respective tissues as previously described by us[4] in detail. The optimal experimental conditions for the study were determined for amnion and chorion and found to be similar for the placental cotyledon.[4] Each determination was performed in duplicate. The radioactive steroids, [7-^{3}H(N)]pregnenolone and [4-^{14}C]progesterone were purchased from New England Nuclear (Boston, Mass.) and had a purity of greater than 99%. They were checked further for purity by thin layer chromatography and high-pressure liquid chromatography. The mitochondrial content for each assay tube was 2.5 to 3.5 mg/protein/ml, and 0.05 μCi of [7-^{3}H(N)]pregnenolone (specific activity, 19.3 Ci/mmol) was added. The substrate concentration used was arrived at by titrating increasing concentrations of tritiated pregnenolone with a constant amount (2.5 to 3.5 mg) of mitochondrial preparation. The concentration of tritiated pregnenolone, which gave the maximum conversion to progesterone, was then used for all assays. Incubation was carried out in a shaking water bath at 37° C for 30 minutes. The reaction was terminated by placing the tubes in an ice bath, and 0.03 μmol each of unlabeled progesterone and pregnenolone, and about 10,000 cpm of [4-^{14}C]progesterone (specific activity, 57.2 mCi/mmol) were added. After mixing and allowing equilibration for 5 minutes, the mixture was extracted twice with diethyl ether:acetyl acetate (1:1, by volume). The combined organic extract was dried and subjected to thin layer chromatography on silica gel plates with chloroform:acetone:ethanol (84:15:1, by volume). Unlabeled progesterone and pregnenolone served as reference standards. Progesterone appeared as a dark absorbed spot under wavelength ultraviolet light and its R_f, relative to the solvent front, was 0.51. Pregnenolone, which appeared as a bluish red spot after spraying with 10% alcoholic phosphomolybdic acid and heated for 1 minute at 100° C, had an R_f, relative to the solvent front, of 0.38. The spots corresponding to progesterone and pregnenolone were cut out, put into scintillation vials to each of which 8 ml of scintillation fluid was added, and then counted for tritium-labeled and carbon 14–labeled standards. Equivalent amounts of tritiated pregnenolone and carbon 14–progesterone as originally used were counted for their radioactivity. The enzymatic activity was calculated from the conversion of tritiated pregnenolone to tritiated progesterone, which was corrected for the methodologic losses and subtraction of the blank. The recovery of [4-^{14}C]progesterone was 88% to 92% when incubated with inactivated mitochondria obtained by boiling the mitochondrial fractions. In preliminary experiments, the purity of the isolated progesterone formed was established by reverse isotope dilution. Blanks were determined from experimental tubes containing the solutions used for the reactions but without adding any tissue fractions and the substrate tritiated pregnenolone. At the end of the incubation period, the mixture was subjected to thin layer chromatography, and the spot corresponding to progesterone was eluted and counted for tritium-labeled radioactivity. This was used as the blank to subtract from the radioactivity corresponding to progesterone in the tubes containing

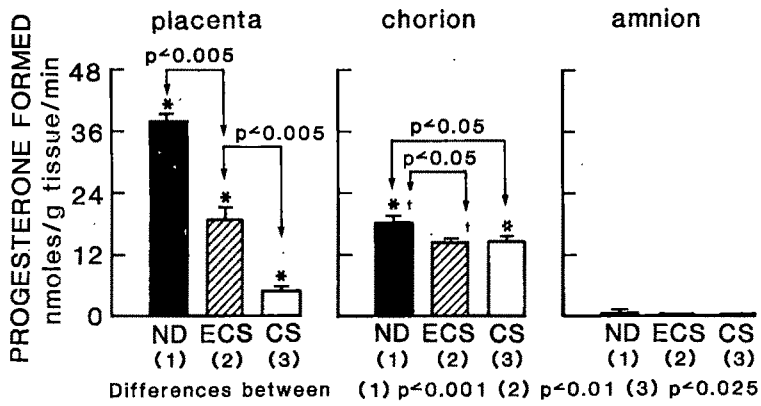


Fig. 2. Mean ± SEM of the in vitro conversion rate of pregnenolone to progesterone in placental tissue, chorion, and amnion from patients with spontaneous normal labor (n = 6) and delivery and from patients undergoing cesarean section after established labor (n = 3) or cesarean section without labor (n = 3). (*ND* = Normal delivery; *ECS* = cesarean section after onset of labor; *CS* = elective cesarean section without labor.)

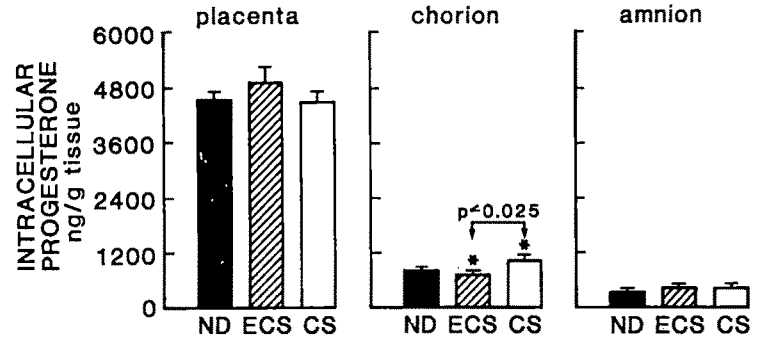


Fig. 3. Intracellular (cytosol) concentrations of progesterone (mean ± SEM) in placental tissue, chorion, and amnion from patients with normal labor and delivery (n = 6) and from patients undergoing cesarean section without labor (n = 3) or cesarean section without labor (n = 3). (*ND* = Normal delivery; *ECS* = cesarean section after onset of labor; *CS* = elective cesarean section without labor.)

tissue mitochondria. The blanks were consistently less than 1% of the substrate used in the experiment tubes. All results were corrected for blanks.

Protein content for the mitochondrial preparation was determined by the method of Lowry et al.[5] with bovine serum albumin as the standard.

Cytosol progesterone. Intracellular progesterone concentrations were determined in the cytosol fraction by a sensitive and specific radioimmunoassay[6] using progesterone antiserum raised in rabbits immunized against progesterone-11–oxime bovine serum albumin conjugate. All results were corrected for procedural losses.

Statistical/analysis. All comparisons of means ± SEM of conversion rates of pregnenolone to progesterone in similar tissues between different groups of patients were analyzed by means of the nonpaired Student *t* test. Differences between different tissues of the same group of patients were analyzed by the paired Student *t* test.

Results

Fig. 2 shows that the rate of pregnenolone to progesterone in placentas from normal delivery (38.7 ± 1.3 nmol/min/gm of tissue) was significantly higher than in cesarean section after labor (19.1 ± 0.50 nmol/min/gm of tissue) or elective cesarean section without labor (5.1 ± 1.8 nmol/min/gm of tissue) (p = <0.005). Similarly, the chorion biosynthesized more progesterone from pregnenolone in normal delivery (19.7 ± 1.1 nmol/min/gm of tissue) than in cesarean section after labor (14.1 ± 1.2 nmol/min/gm of tissue) or cesarean section without labor 14.7 ± 0.4 nmol/min/gm of tissue) (p = <0.05). The amnion had little or undetectable conversion of pregnenolone to progesterone in all three clinical types of delivery situations. The placenta

was significantly faster than the chorion or amnion in the bioconversion of pregnenolone to progesterone in patients with normal delivery ($p = <0.001$).

Progesterone concentrations in the cytosol of placental tissue, chorion, and amnion are shown in Fig. 3. The mean ± SEM progesterone concentration in the placenta was 4590 ± 137 ng/gm of tissue after normal delivery, 4465 ± 336 ng/gm of tissue after cesarean section with labor, and 4496 ± 228 ng/gm of tissue after cesarean section without labor. The corresponding progesterone concentrations in the chorion were 897 ± 25, 813 ± 26, and 933 ± 21 ng/gm of tissue; in the amnion they were 399 ± 16, 453 ± 35, and 448 ± 19 ng/gm of tissue. In all subjects, cytosol progesterone concentrations were significantly higher in the placenta than in the chorion or amnion ($p = <0.001$).

Comment

The importance of progesterone as a key regulator in the endocrine and biochemical cascade leading to uterine activation and parturition has been strongly supported by Csapo.[7] In the present study, an attempt was made to examine the role of local progesterone content and its biosynthesis reserve capacity within the placenta and its membranes during spontaneous labor. It is clear that there are significantly higher concentrations of progesterone within the placental cotyledon than within either the chorion or the amnion on a weight-for-weight basis. The placenta has about five times higher progesterone concentration than the chorion and about 10 to 12 times higher than the amnion. With the actual placental cotyledon, there is no apparent significant changes when labor ensued. However, in the chorion, there is significantly less tissue progesterone when labor begins with no futher change, irrespective of the second stage of labor. This suggests that locally there is a fall in the concentration of progesterone within the chorionic membrane tissue immediately before or with the onset of labor. A fall in local progesterone within the chorion can lead to labilization of the lysosomes with release of phospholipase A_2, which causes generation of arachidonic acid and prostaglandin biosynthesis. The chorion has been shown to have a high capacity for prostaglandin synthesis and is thought to be the major source of prostaglandins found in the amniotic fluid.[8] An alternative effect of the falling progesterone concentration in the chorion is to generate the precursor fatty acid, arachidonic acid, which is shuttled to the decidua to be transformed into prostaglandins.[9]

In vitro bioconversion of pregnenolone to progesterone indicates that in placental cotyledon, the rate of progesterone formation is significantly enhanced with the onset of spontaneous labor and further increased after the second stage of labor and vaginal delivery. This suggests that a labor-associated mechanism or factor activates the enzyme complex 5-ene-3β-hydroxysteroid dehydrogenase and 5-ene-4-ene isomerase. This may initially appear paradoxic with respect to progesterone withdrawal and onset of labor. However pregnenolone-progesterone conversion gives a measure of the tissue enzyme activity and capacity for progesterone biosynthesis from its precursor, pregnenolone. Furthermore, the placental tissue concentrations of progesterone do not show any significant difference with or without labor. Although the enzymes for pregnenolone-progesterone conversion may be activated with labor, other factors such as reduced precursor availability, local in vitro factors inhibiting progesterone formation, or increased progesterone metabolism may account for the absence of changes in tissue progesterone. Progesterone levels in the uterine vein draining from the placenta have been found to decline significantly with labor, thus indirectly suggesting reduced placental progesterone output.[7]

Several studies, including those from our laboratory,[4, 9-11] have indicated that steroids such as estrone, estradiol, estriol, testosterone, dehydroepiandrosterone sulfate, and progesterone can inhibit local placental progesterone biosynthesis. Steroids such as estradiol and estriol, as well as the C_{19} androgens, are produced in the trophoblast cells of the placenta during aromatization of precursor steroids transferred from the fetus. During pregnancy, placental production and secretion of estradiol and estriol increase until term and therefore could possibly exert retroinhibition of the enzyme 5-ene-3B-hydroxysteroid dehydrogenase that leads to reduced placental production of progesterone in vivo. However, when the inhibiting steroids are removed such as in the in vitro experiments in the present study, the ability of the placental cotyledon to produce progesterone is not inhibited.

Our findings indicate that the amnion converts hardly any pregnenolone to progesterone but the chorion does. The chorion obtained after the second stage of labor (delivery) converts significantly more pregnenolone to progesterone than chorion obtained after elective cesarean section or cesarean section during the first stage of labor. After the onset of labor, tissue progesterone concentrations are reduced, but no further change occurs after the second stage of labor. This suggests that the progesterone produced is locally metabolized or converted within the tissues, since progesterone levels in the uterine vein[7] decline with labor. Although the placental tissue has increasing 20-hydroxysteroid oxidoreductase enzyme activity and therefore increasing rates of progesterone metabolism with advancing gestation,[12] the fetal membranes decrease their rates of progesterone metabolism at term.[13]

Within the fetal membranes, progesterone-binding substance[14] binds progesterone and thus reduces progesterone concentration of free tissue. A fall in progesterone levels may cause labilization of lysosomes and release of the lysosomal enzyme, phospholipase A_2, leading to initiation of the arachidonic acid casade and prostaglandin production.

The present findings indicate labor-related reduction in progesterone production from pregnenolone in the placenta and chorion but decreased progesterone concentration in the chorionic tissue with the onset of labor, suggesting possibly the binding of progesterone to the progesterone-binding substance in the fetal membranes[14] and therefore not measurable. If the metabolism of progesterone and the activity of progesterone-binding substance in the fetal membranes and placenta are taken into consideration, the present findings indicate that progesterone production from its precursor does not appear to be impeded. However, progesterone is taken up for binding with the progesterone-binding substance in the fetal membranes while the placenta can rapidly metabolize progesterone to reduce its availability and drainage out of the uterus. Such a series of mechanisms affecting local progesterone production, binding, and metabolism within the immediate environment of the placenta, fetal membranes, and uterus is consistent with and lend support to the concept that local withdrawal of progesterone plays an important role in the onset of human labor.

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Metabolism of lipoproteins by human fetal hepatocytes

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The rate of clearance of lipoproteins from plasma appears to play a role in the development of atherogenesis. The liver may account for as much as two thirds of the removal of low-density lipoprotein and one third of the clearance of high-density lipoprotein in certain animal species and humans, mainly by receptor-mediated pathways. The purpose of the present investigation was to determine if human fetal hepatocytes maintained in vitro take up and degrade lipoproteins. We first determined that the maximal binding capacity of iodine 125–iodo-LDL was approximately 300 ng of low-density lipoprotein protein/mg of membrane protein and an apparent dissociation constant of approximately 60 μg low-density lipoprotein protein/ml in membranes prepared from human fetal liver. We found that the maximal uptake of [^{125}I]iodo-LDL and [^{125}I]iodo-HDL by fetal hepatocytes occurred after 12 hours of incubation. Low-density lipoprotein uptake preceded the appearance of degradation products by 4 hours, and thereafter the degradation of low-density lipoprotein increased linearly for at least 24 hours. In contrast, high-density lipoprotein was not degraded to any extent by fetal hepatocytes. [^{125}I]Iodo-LDL uptake and degradation were inhibited more than 75% by preincubation with low-density lipoprotein but not significantly by high-density lipoprotein, whereas [^{125}I]iodo-HDL uptake was inhibited 70% by preincubation with high-density lipoprotein but not by low-density lipoprotein. In summary, human fetal hepatocytes take up and degrade low-density lipoprotein by a receptor-mediated process similar to that described for human extrahepatic tissues. (Am J Obstet Gynecol 1987;157:1338-44.)

Key words: Low-density lipoprotein, high-density lipoprotein, liver, human, fetal

The clearance of lipoprotein cholesterol from plasma appears to play a role in the development of atherogenesis in humans. Circulating low-density lipoproteins are removed from plasma by a combination of receptor-dependent and receptor-independent mechanisms by various tissues in many animal species.[1] Most investigators have reported that the receptor-dependent mechanisms of low-density lipoproteins account for 56% to 80% of the low-density lipoprotein turnover in various animal species[1, 2] and humans.[3] Although the adrenal gland is the organ in which the rate of low-density lipoprotein catabolism is the greatest on a tissue weight basis, the liver, by virtue of its size, is the major site of low-density lipoprotein catabolism in rats, swine, rabbits, and also humans.[2] The mechanism of uptake and metabolism of high-density lipoproteins is less well understood, but the adrenal, gonads, and liver are believed to be the principal sites of clearance of high-density lipoproteins.[4]

Very little information is available on the metabolism of lipoproteins by human liver cells. In a preliminary study Brown et al.[5] reported that human fetal liver membrane preparations demonstrated saturable binding for low-density lipoproteins. The affinity and maximal binding were less than that observed in human fetal adrenal glands and testes. Hoeg et al.[6] recently reported that the affinity for low-density lipoproteins was less in human adult livers compared with fibroblasts. The purpose of the present investigation was to determine if human fetal hepatocytes maintained in vitro would take up and degrade lipoproteins and to determine if the mechanisms were similar to that observed for extrahepatic human tissues.

Material and methods

Liver tissue. Liver tissue was obtained from abortuses of 10-24 weeks' gestation delivered by induced abortion (dilatation and extraction). Tissues were obtained in accordance with the Donors Anatomical Gift Act of the State of Texas after written consent was obtained from the woman to be aborted. A consent form and protocol approved by the Institutional Review Board of the University of Texas Health Science Center at Dallas were used.

Preparation and culture of hepatocytes. Human fetal hepatocytes were prepared and characterized as described previously.[7] The cells were maintained in primary culture in medium containing arginine-deficient Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, N.Y.) to suppress fibroblast

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growth plus fetal calf serum (10% v/v) and antibiotics (penicillin, 1000 U/ml; amphotericin B [Fungizone], 2.5 μg/ml; and streptomycin, 1000 μg/ml) (Grand Island Biological Co.) for 24-48 hours. Thereafter cells were maintained in the presence or absence of various lipoproteins as discussed in the Results section.

Lipoproteins. Low-density lipoproteins, (density, 1.019 to 1.063 gm/ml), HDL_3 (density, 1.125 to 1.215 gm/ml), and lipoprotein-poor serum were prepared as previously described.[8] Iodine 125–iodo-LDL and [^{125}I]iodo-HDL were prepared as previously described.[9] The specific activity of [^{125}I]iodo-LDL and [^{125}I]iodo-HDL ranged from 250 to 400 cpm/ng of protein.

Lipoprotein uptake by human fetal hepatocytes. The cells were maintained in culture as previously described on day 2 or 3 of culture, the media was changed to lipoprotein-poor serum for 3 additional days. On the fifth or sixth day of culture, the cells were rinsed twice with 0.15 mol/L of NaCl. One milliliter of fresh medium containing lipoprotein-poor serum (10%, v/v) and [^{125}I]iodo-LDL or [^{125}I]iodo-HDL was added to each dish. Incubation was continued at 37° C in a humidified atmosphere of 95% air and 5% CO_2. At the end of the incubation period, the dishes were removed from the incubator and placed on ice. The medium was removed, and the cells were rinsed three times with 0.15 mol/L of cold NaCl. The cells were scraped and homogenized in 1 ml 0.15 mol/L of NaCl; an aliquot (500 μl) of the homogenate was assayed for radioactivity. Duplicate aliquots (100 μl) were assayed for protein content.

Degradation of lipoproteins by human fetal hepatocytes. To measure the degradation of [^{125}I]iodo-LDL or [^{125}I]iodo-HDL to amino acids (monoiodotyrosine), the culture medium of cells incubated in the presence of [^{125}I]iodo-LDL or [^{125}I]iodo-HDL was removed from each dish and mixed with 1 ml of trichloroacetic acid (50%, wt/vol). Three milliliters of bovine serum albumin (5 mg·ml^{-1}) were added to the acidified culture medium, and the mixture was allowed to stand for 30 minutes at 4° C. The tubes were then centrifuged at 1500 rpm for 5 minutes. An aliquot (3 ml) of supernatant fluid was mixed with 30 μl potassium iodide solution (40%, w/v) and 120 μl of H_2O_2 (30%). After standing at room temperature for 5 minutes, the mixture was extracted with 10 ml of $CHCl_3$ to remove the free iodine, and 2 ml aliquots of the aqueous layer were assayed for radioactivity. The radioactivity contained in trichloroacetic acid–soluble substances in the culture medium incubated with [^{125}I]iodo-LDL or [^{125}I]iodo-HDL in the absence of tissue was assayed. This value served as the blank and was used in the calculation of the results.

Results are expressed as nanograms of [^{125}I]iodo-LDL or [^{125}I]iodo-HDL taken up or degraded per milligram of protein per unit time, and each data point represents the mean ± SE of four replicate dishes or the average of duplicate dishes as described in the figure legends.

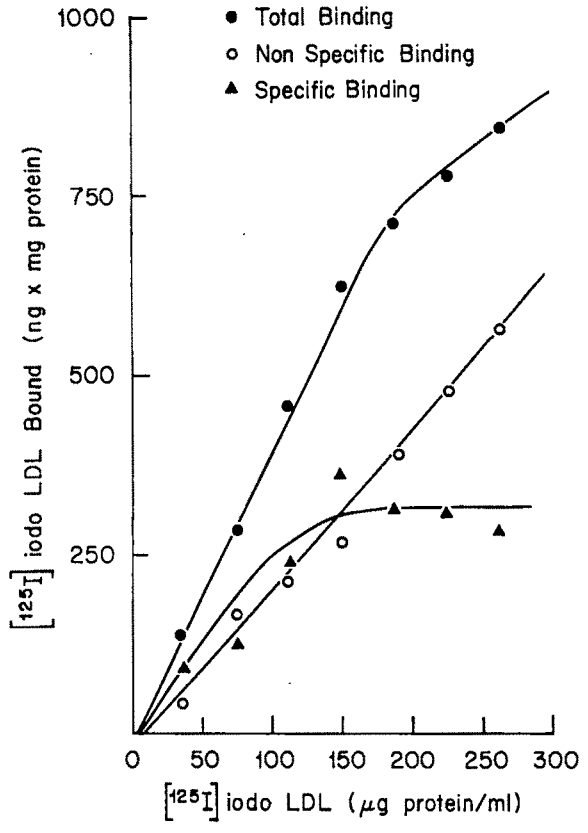


Fig. 1. The total (●), nonspecific (○), and specific binding (▲) of [^{125}I]iodo-LDL to human fetal liver membrane fractions as a function of [^{125}I]iodo-LDL concentration. Each data point represents the average of duplicate incubations.

[^{125}I]Iodo-LDL binding in human fetal liver membrane preparations. Fresh human fetal liver membrane preparations were prepared as described previously for adrenal tissues.[10] The binding of [^{125}I]iodo-LDL to liver membrane preparations was assayed as previously described for human fetal adrenal membrane preparations.[10]

Chemicals. Bovine serum albumin and trichloroacetic acid were obtained from Sigma Chemical Co. (St. Louis, Mo.). Other chemicals used were of analytic grade.

A statistical analysis of the results was performed by means of an analysis of variance followed by Newman-Keuls multiple comparison analysis. Each figure (2 through 6) was repeated two to three times and a representative experiment is presented.

Results

We first determined whether specific binding sites for low-density lipoproteins were present in membrane fractions prepared from human fetal liver tissue. The specific binding of [^{125}I]iodo-LDL as a function of the

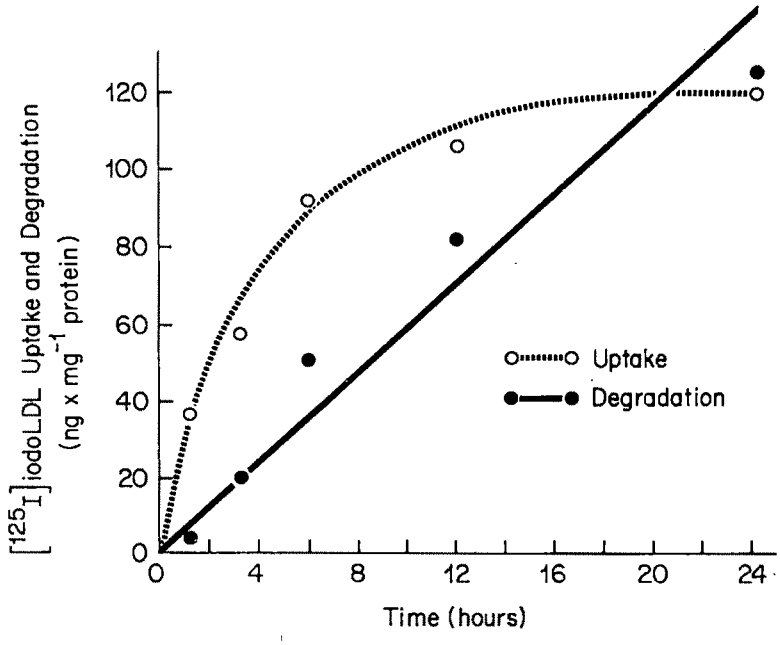


Fig. 2. Time course of uptake and degradation of [^{125}I]iodo-LDL by human fetal hepatocytes. Cells were maintained for 72 hours in the presence of medium containing lipoprotein-poor serum. Thereafter, [^{125}I]iodo-LDL (10 μg of low-density lipoprotein protein/ml) was added at time 0. Each data point represents the average of duplicate incubations.

concentration of [^{125}I]iodo-LDL in the incubation medium is presented in Fig. 1. The specific binding was computed from the difference between total binding and binding observed in the presence of excess nonradiolabeled low-density lipoprotein. From the results of these experiments, we suggest that the binding of [^{125}I]iodo-LDL is saturable, with a maximal binding capacity of approximately 300 ng of low-density lipoprotein protein/mg of membrane protein (range, 190 to 372 ng/mg; n = 9) and an apparent dissociation constant of approximately 60 μg of low-density lipoprotein protein/ml (range, 60 to 344 ng/ml; n = 9) as determined by Scatchard plot.

The uptake and degradation of [^{125}I]iodo-LDL by human fetal hepatocytes as a function of time are presented in Fig. 2. Human fetal liver cells were maintained for 72 hours at 37° C in the absence of lipoproteins lipoprotein-poor serum. [^{125}I]Iodo-LDL (10 μg of low-density lipoprotein protein/ml) was added to the culture medium at time 0. Thereafter, the rate of uptake of [^{125}I]iodo-LDL increased for 12 hours, and no further uptake was noted during the 24-hour period. Degradation of [^{125}I]iodo-LDL was detected by 1 hour and continued to increase in a linear fashion for at least 24 hours.

The effect of temperature on the uptake and degradation of [^{125}I]iodo-LDL by human fetal hepatocytes was also assessed. Cells were maintained for 72 hours at 37° C in media containing lipoprotein-poor serum. Thereafter, cells were maintained at 4° C for 1 hour, and [^{125}I]iodo-LDL (20 μg of low-density lipoprotein protein/ml) was added to the culture medium. Maximal cellular uptake of [^{125}I]iodo-LDL at 4° C was attained by 25 minutes and did not increase further (data not shown). In contrast, the degradation of [^{125}I]iodo-LDL was markedly inhibited, a finding indicating that the degradation process is temperature dependent.

Next, the effect of preincubation of human fetal hepatocytes with various concentrations of nonradiolabeled low-density lipoprotein or high-density lipoprotein on the uptake (Fig. 3) and degradation (Fig. 4) of [^{125}I]iodo-LDL was investigated. Cells were maintained for 72 hours in the presence or absence of nonradiolabeled lipoprotein. Thereafter [^{125}I]iodo-LDL (10 μg of low-density lipoprotein protein/ml) was added to the culture medium for 6 hours. Low-density lipoproteins significantly inhibited [^{125}I]iodo-LDL uptake and degradation in a dose-related fashion (Figs. 3 and 4). Both degradation and uptake were inhibited significantly by 25 μg of low-density lipoprotein protein/ml. In contrast, high-density lipoproteins did not inhibit the uptake of [^{125}I]iodo-LDL in concentrations of up to 500 μg of high-density lipoprotein protein/ml (Fig. 3). The presence of high-density lipoproteins in the preincubation medium also had a minimal influence on [^{125}I]iodo-LDL degradation (Fig. 4).

The uptake and degradation of [^{125}I]iodo-HDL by human fetal hepatocytes as a function of time are pre-

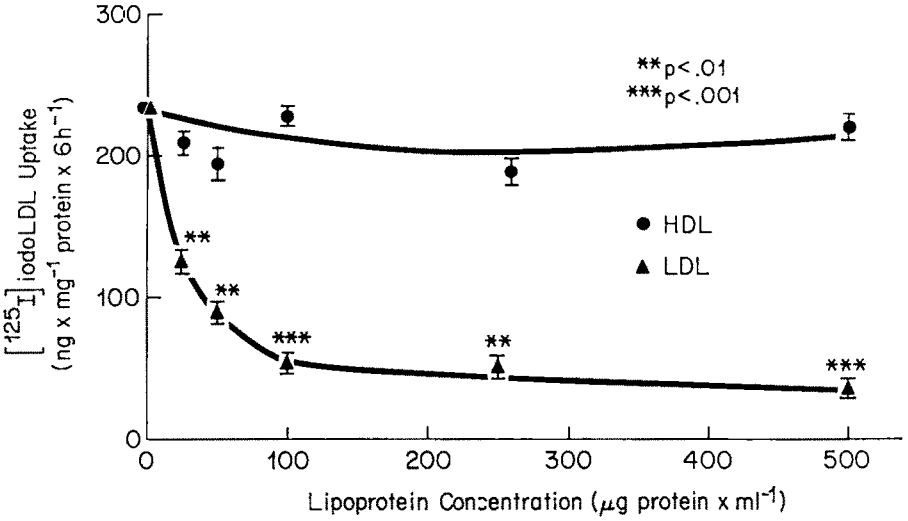


Fig. 3. The effect of lipoproteins on the rate of uptake of [^{125}I]iodo-LDL by human fetal hepatocytes. Cells were maintained for 72 hours in the presence of various concentrations of nonradiolabeled low-density lipoprotein or high-density lipoprotein. Thereafter, the cells were rinsed with fresh medium containing lipoprotein-poor serum and [^{125}I]iodo-LDL (10 μg of low-density lipoprotein protein/ml) was added to the culture medium for 6 hours. Each data point represents the mean and SE of quadruplicate dishes. (*HDL* = High-density lipoprotein; *LDL* = low-density lipoprotein.)

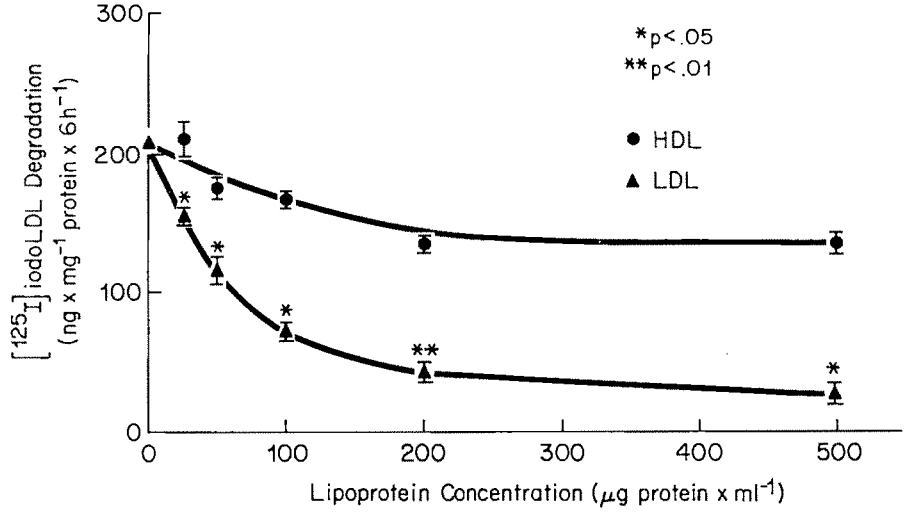


Fig. 4. The effect of lipoproteins on the rate of degradation of [^{125}I]iodo-LDL by human fetal hepatocytes. Cells were maintained for 72 hours in the presence of various concentrations of nonradiolabeled low-density lipoprotein or high-density lipoprotein. Thereafter, the cells were rinsed with fresh medium containing lipoprotein-poor serum and [^{125}I]iodo-LDL (10 μg of low-density lipoprotein protein/ml) was added to the culture medium for 6 hours. Each data point represents the mean and SE of quadruplicate dishes. (*HDL* = High-density lipoprotein; *LDL* = low-density lipoprotein.)

sented in Fig. 5. Cells were maintained at 37° C for 72 hours in medium containing lipoprotein-poor serum. Thereafter, [^{125}I]iodo-HDL (10 μg of high-density lipoprotein protein/ml) was added to the culture medium. The uptake of [^{125}I]iodo-HDL increased with time for 12 hours and remained constant for as long as 24 hours. In contrast, the degradation of [^{125}I]iodo-HDL was extremely low.

Finally, we investigated the effect of nonradiolabeled lipoprotein on the rate of uptake of [^{125}I]iodo-HDL by human fetal hepatocytes. Cells were maintained at 37° C in the presence of various concentrations

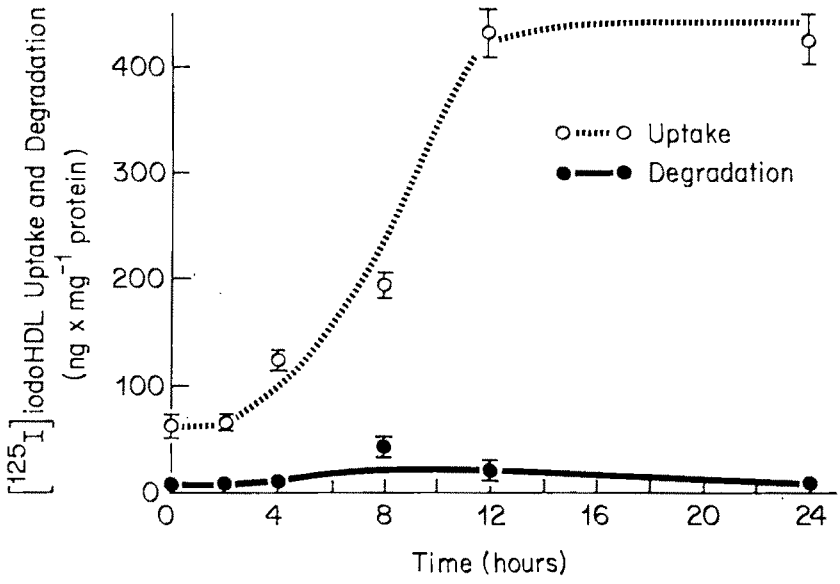


Fig. 5. Time course of the metabolism of [^{125}I]iodo-HDL by human fetal hepatocytes. Cells were maintained for 72 hours in the presence of lipoprotein-poor serum. Thereafter, [^{125}I]iodo-HDL was added to the culture medium (10 μg of high-density lipoprotein protein/ml) for 24 hours. Each data point represents the mean and SE of quadruplicate dishes.

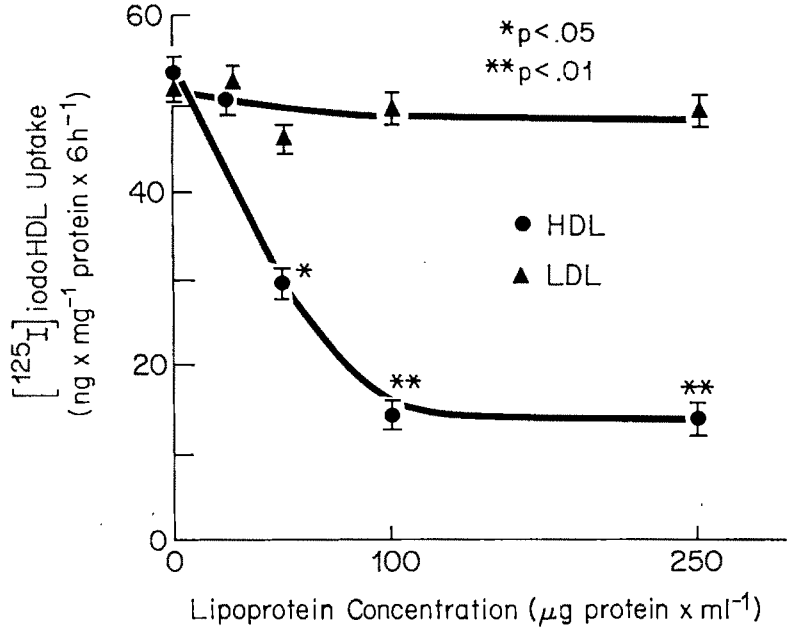


Fig. 6. The effect of lipoproteins on the uptake of [^{125}I]iodo-HDL by human fetal hepatocytes. Human fetal hepatocytes were maintained for 72 hours in the presence of various concentrations of nonradiolabeled high-density lipoprotein or low-density lipoprotein. Thereafter, the cells were rinsed with fresh medium containing lipoprotein-poor serum and [^{125}I]iodo-HDL (10 μg of high-density lipoprotein protein/ml) was added to the culture medium for 6 hours. Each data point represents the mean and SE of quadruplicate dishes. (*HDL* = High-density lipoprotein; *LDL* = low-density lipoprotein.)

of nonradiolabeled low-density lipoprotein or high-density lipoprotein for 72 hours. Thereafter, the medium was changed, and [^{125}I]iodo-HDL (10 μg of high-density lipoprotein protein/ml) was added to the culture medium for an additional 6 hours (Fig. 6). Preincubation of fetal hepatocytes with low-density lipoprotein up to 500 μg of low-density lipoprotein protein/ml had no effect on the uptake of [^{125}I]iodo-HDL

by fetal hepatocytes. In contrast, preincubation with nonradiolabeled high-density lipoproteins inhibited uptake of [^{125}I]iodo-HDL in a dose-related fashion.

Comment

In a previous investigation, we studied the effect of lipoproteins on cholesterol synthesis by human fetal hepatocytes maintained in culture.[11] We observed that low-density lipoproteins inhibited cholesterol synthesis in a dose-related fashion, which suggested that low-density lipoproteins were acting via a receptor-mediated process similar to that described for extrahepatic human fetal tissues. In the present study, we observed that human fetal liver membrane preparations exhibited saturable binding for [^{125}I]iodo-LDL. Although the affinity for low-density lipoproteins was low in human fetal lever membranes, these results were similar to those reported by Brown et al.,[5] who previously reported evidence for a binding site for low-density lipoproteins on human fetal liver membrane preparations, and by Hoeg et al.[6] in adult human liver membrane preparations. In contrast, one report demonstrated that the human adult liver contains only the presence of apolipoprotein E receptors but not apolipoprotein B receptors. Furthermore, these investigators reported that there was no significant binding of low-density lipoproteins to human adult membranes.[12] The reasons for the lack of binding of low-density lipoproteins in that study of adult liver membranes in vitro are unclear. Recently, Bilheimer et al.[3] reported that the transplantation of a liver from a normal child to a subject with homozygous familial hypercholesterolemia with defective and reduced low-density lipoprotein receptors was followed by rapid decline in low-density lipoprotein–cholesterol plasma levels and an increase in cholesterol catabolic rate of intravenously administered [^{125}I]iodo-LDL. These results suggest that the livers of adult, children, and fetuses contain receptors for low-density lipoprotein.

In the present study, we have shown that human fetal hepatocytes take up and degrade [^{125}I]iodo-LDL in a manner similar to that described for extrahepatic tissues in the human fetal adrenal, placenta, and adult corpus luteum and fibroblasts.[13] The uptake of low-density lipoproteins is followed by internalization by a process of adsorptive endocytosis. The vesicles formed by endocytosis apparently fuse with lysosomes and the protein compartment is hydrolyzed to amino acids whereas the cholesterol esters are hydrolyzed to fatty acids and cholesterol, as also reported to occur in extrahepatic tissues. The cholesterol formed may function by inhibiting endogenously formed cholesterol[11] or be reused in the formation of lipoproteins and then excreted by the liver.

We observed that nonradiolabeled low-density lipoproteins clearly inhibited [^{125}I]iodo-LDL uptake and degradation by human fetal hepatocytes in a dose-related fashion. In contrast, apolipoprotein E–free high-density lipoproteins had no significant effect on low-density lipoprotein uptake or degradation. These results were different than those reported to occur with pig hepatocytes.[14] In that study apolipoprotein E–free high-density lipoproteins inhibited low-density lipoprotein uptake but to a lesser degree than did low-density lipoproteins.

The mechanism for the regulation of the metabolism of high-density lipoproteins by cells is less clear. Evidence from our laboratory previously suggests that the human HDL_3 fraction, which primarily contains apoprotein AI and AII but no apoprotein E, stimulated cholesterol synthesis in human fetal hepatocytes.[11] Others have reported that high-density lipoprotein has the capacity to remove unesterified cholesterol from cells in culture.[2] The nature of cellular interaction and binding, as well as catabolism of high-density lipoprotein, is presently unclear.

In the present investigation, we have demonstrated that [^{125}I]iodo-HDL is taken up by human fetal hepatocytes as a function of time as was true for human fetal adrenal tissue and that [^{125}I]iodo-HDL degradation occurred at an insignificant rate as was also observed for human fetal adrenal tissue.[9] Others have reported that high-density lipoprotein binds to placental and testicular tissue and fibroblasts but that degradation was negligible.[4] In contrast, high-density lipoprotein was apparently internalized and the degradation of high-density lipoprotein was inhibited by chloroquine by cultured hepatocytes of rats and pigs.[14, 15] Utilizing pig hepatocytes, Bachorik et al.[14] reported that both apolipoprotein E–free high-density lipoprotein uptake and degradation was inhibited by both high-density lipoprotein and low-density lipoprotein. In our investigation using human fetal hepatocytes, the uptake of [^{125}I]iodo-HDL was inhibited by preincubation with high-density lipoprotein but not by low-density lipoprotein. Recently, Hoeg et al.[16] characterized high-density lipoprotein binding to human adult liver membranes but did not investigate the uptake and degradation in normal adult liver cells.

In conclusion, human fetal hepatocytes take up and degrade low-density lipoprotein in a similar fashion to that for human extrahepatic tissues. The uptake of low-density lipoprotein appears to be a receptor-mediated process, and the receptors recognizing low-density lipoprotein and high-density lipoprotein are different. Human fetal hepatocytes also take up apolipoprotein E–free high-density lipoprotein but do not degrade it, and the role of the fetal liver with respect to the clearance of high-density lipoprotein is less clear.

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The prevalence of autoantibodies in pregnant and nonpregnant women

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The frequency of autoantibodies during pregnancy was studied. Sera from 136 women (84 pregnant and 52 nonpregnant) were tested for antibodies directed against nuclear antigens, smooth muscle antigens, gastric parietal cells, mitochondria, and striated muscle by indirect immunofluorescent microscopy. Rheumatoid factor was measured by a latex agglutination test, and thyroid microsomal and thyroglobulin antibodies were measured by a hemagglutination assay. Immunofluorescence assays were considered positive if antibodies were detected at a serum dilution of 1:20. All positive samples were retested at increasing dilutions until immunofluorescence or agglutination could not be detected. The control population consisted of 52 normal, healthy, ovulatory, and nonpregnant women. The study population consisted of 84 pregnant women, none of whom had a known autoimmune disorder. The mean ages of the two groups were 32 and 26 years, respectively. The frequency of autoantibody detection was not significantly different in comparing the study (46.2) and control groups (40.5). The results did not support the hypothesis that pregnancy has an effect on the induction or suppression of autoantibody production. Previous studies that suggested differences may have been influenced by the lack of a control group. (Am J Obstet Gynecol 1987;157:1345-50.)

Key words: Autoantibodies, pregnancy, immunology

There has been little agreement on the effect of pregnancy on the production of autoantibodies. Both an increase and a decrease have been reported[1, 2] in patients with autoimmune disease. Previous studies have generally examined autoantibodies in patients with known autoimmune disorders or who had medical complications related to pregnancy. Few studies have examined autoantibodies in uncomplicated pregnancies of healthy women.

Autoantibodies have been detected in "normal" pregnant subjects[3-5] and appear to be more frequent than in nonpregnant control groups. Yet the significance of autoantibody detection during pregnancy remains unclear. One difficulty in the interpretation of previous studies is that changes in only a single autoantibody are generally reported, and a larger survey of autoantibody production during pregnancy has never been conducted. A more critical problem in many earlier reports was the lack of an adequate control group. The purpose of this study was to determine the frequency of several autoantibodies in a pregnant population and a nonpregnant control group. Medical, family, gynecologic, and reproductive histories were reviewed to define the study population adequately and determine if any historical factors were related to the presence or absence of autoantibodies.

From the Departments of Obstetrics and Gynecology and Medical Statistics and Epidemiology, Mayo Clinic.
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Reprint requests: Phillip E. Patton, M.D., Department of Obstetrics and Gynecology, Oregon Health Sciences Center, Portland, OR 97201.

Material and methods

Subjects. Sera from a total of 136 women seen in the Department of Obstetrics and Gynecology at the Mayo Clinic from March 1983 to October 1984 were tested for autoantibodies. The study population consisted of 84 consecutive low-risk pregnant subjects admitted with uncomplicated pregnancies, and all but two had blood drawn in the third trimester. Ages ranged from 15 to 36 years, with a mean of 26 years. The nonpregnant control population consisted of 52 paid volunteers without major systemic illnesses or a known autoimmune disease. All subjects used barrier contraception and were ovulatory on the basis of basal body temperature charting or serum progesterone assay. Ages ranged from 19 to 48 years, with a mean age of 32 years. No subject in the study had a history of autoimmune thyroiditis, pernicious anemia, systemic lupus erythematosus, or other known autoimmune disease.

Histories in both control and pregnant subjects were carefully abstracted for significant medical illness, familial disease, and gynecologic and reproductive disorders. Past and current pregnancies were reviewed for outcome (abortion, stillbirth, and viable term delivery) and intrapartum and postpartum complications. Fetal

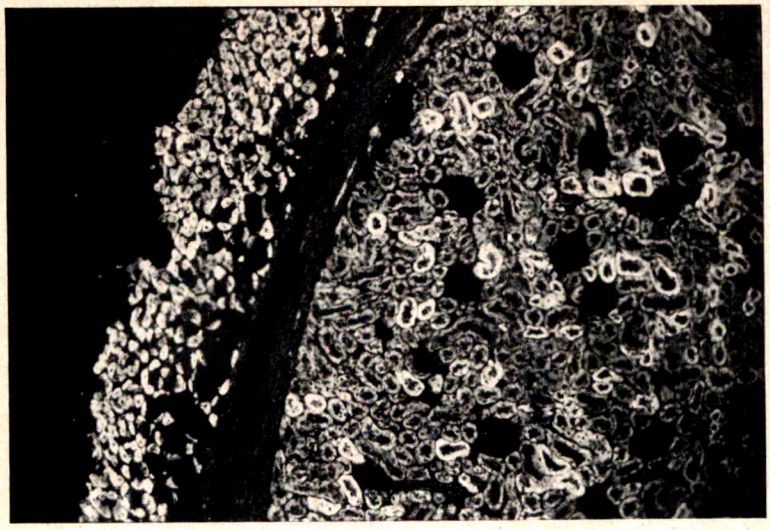


Fig. 1. Positive immunofluorescence of gastric parietal cells and kidney mitochondria.

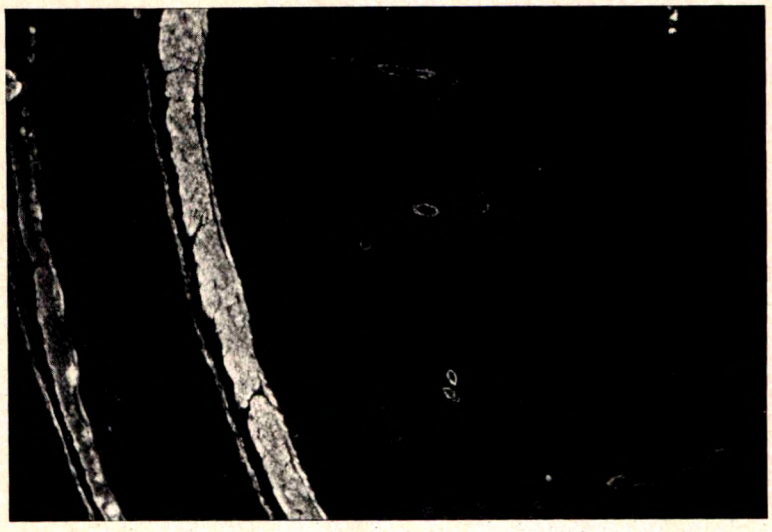


Fig. 2. Positive immunofluorescence of gastric smooth muscle.

weight, Apgar score, morbidity, and mortality were reviewed for both past and current pregnancies.

Indirect immunofluorescence. Sera were stored at −20° C until assayed. Antibodies directed against nuclei, smooth muscle, striated muscle, gastric parietal cells, and mitochondria were assayed by indirect immunofluorescent microscopy. Unfixed cryosections (4 to 5 μm) mounted on slides were incubated with a 1:20 to 1:60 dilution of the patient's serum in phosphate-buffered saline solution for 30 to 45 minutes at 22° C. Sera from patients with a documented autoimmune disease served as positive controls. A negative control (normal male) and diluent buffer control were also run in each assay. Fluorescein-conjugated goat polyvalent antisera against human immunoglobulins (IgG, IgM, κ, and λ) was the second antibody. Slides were graded on a scale of 0 to + + + + (0 equaled background immunofluorescence and + + + + equaled the fluorescence intensity of the positive control). Each slide was graded independently and blindly by two observers.

Samples were considered positive if immunofluorescence was detected at a 1:20 dilution. All positive sera were retested at increasing dilutions until immunofluorescence could not be detected.

Agglutination assays. Sera were screened for rheumatoid factor by use of a latex agglutination test (Rheumanosticon:Organon). A 1:20 or greater dilution of sera in phosphate-buffered saline solution was tested according to kit instructions.

Thyroid microsomal and thyroglobulin antibodies

Table I. Single occurrence of autoantibodies in pregnant and nonpregnant women (N = 136)

Positive serologic test	*No. of subjects*
A. Thyroglobulin	0
B. Thyroid microsome	14
C. Smooth muscle	7
D. Mitrochondria	0
E. Antinuclear	11
F. Rheumatoid factor	1
G. Gastric parietal cell	9
Joint occurrence of autoantibodies	
A + B	2
A + B + G	2
B + C	2
B + E	4
B + E + F	1
C + G	1
D + G	4
Total	58

Table II. Frequency of autoantibodies in pregnant and nonpregnant women (N = 136)

Autoantibody	*Total positive subjects*	*%*
A. Thyrogobulin	4	2.9
B. Thyroid microsome	25	18.4
C. Smooth muscle	10	7.4
D. Mitrochondrial	4	2.9
E. Antinuclear	16	11.8
F. Rheumatoid factor	2	1.5
G. Gastric parietal cell	16	11.8

were measured by a sensitized red cell agglutination test (Seratek: Ames). A 1:20 or greater dilution of sera in phosphate-buffered saline solution was tested according to kit instructions.

Figs. 1 and 2 are representative examples of positive autoantibodies directed against mitochondria and smooth muscle, respectively.

Statistical analysis. Statistical associations were tested with the χ^2, Fisher's exact (sparse tables), and rank-sum tests as appropriate. In view of the large number of tests preferred, a nominal p value of <0.01 was considered significant to minimize the detection of spurious associations.

Results

The finding of a single autoantibody and more than one autoantibody in the study population is presented in Table I. The finding of more than one autoantibody per subject (seen in 16 subjects) did not occur at a frequency more than expected (14.5 expected) under the assumption that the antibodies are independent. Since autoantibodies directed against mitochondria also react with gastric parietal cells, the possibility that two different autoantibodies were present in sera of subjects with positive autoantibodies directed against mitochondria was not excluded.

The frequency of autoantibodies in the total study population is presented in Table II. The autoantibody detected most frequently was the thyroid microsomal antibody. The finding of autoantibodies in the study population was not known to be associated with a documented autoimmune disease.

The study population was divided into a pregnant group and a control group (nonpregnant) to compare the frequency of autoantibodies in each group. The frequency with which at least one autoantibody was detected in the control (46.2%) and pregnant groups (40.5%) did not differ significantly. When the frequency of the most prevalent autoantibodies (i.e., those found in at least five subjects in the total study population) was compared, there was no significant difference between the two groups (Table III).

Historical events that may have been related to the finding of autoantibodies were examined to determine if any events predominated in one group. Historical events that occurred frequently in the subjects' immediate medical history (at least five times in the total study population) included rheumatoid disease and anemia. Historical events in the maternal family history that occurred frequently were cancer, diabetes mellitus, heart disease, and hypertension. Although the control group had a family history of heart disease, kidney disease, and hypertension more often than the pregnant group, none of these factors was found to be significantly associated with the detection of autoantibodies.

To determine if age and gravidity influenced the finding of autoantibodies, the study population (pregnant and nonpregnant groups) was divided into intervals of age and gravidity at the time that blood was drawn (Table IV). Neither gravidity nor age intervals were associated with the occurrence of autoantibodies. A trend for antibodies directed against nuclei and age was noted, but it was not statistically significant.

Pregnant and control subjects differed with respect to age and prior gravida. To exclude the possibility that age or gravidity may have been a bias, the study and pregnant groups were matched. Thirty of the 54 subjects were matched to a pregnant subject of like age and gravidity. When the frequency of autoantibodies was evaluated for those matched pairs, there remained no significant difference in the frequency of autoantibodies between the groups.

The outcome of prior pregnancies was evaluated with respect to the frequency of autoantibodies. Ninety-seven women had a prior pregnancy outcome (other than a therapeutic abortion) before the time that blood

Table III. Pregnancy status and occurrence of autoantibodies

	Pregnant					
	No (n = 52)		*Yes (n = 84)*			
	Positive				*p value*	
Serologic test	*%*	*(No.)*	*%*	*(No.)*	*χ*	*Fisher exact*
Thyroid microsome	23.1	(12)	15.5	(13)	0.27	0.36
Smooth muscle	9.6	(5)	6.0	(5)	0.43	0.51
Antinuclear	15.4	(8)	9.5	(8)	0.30	0.41
Gastric parietal cell	13.5	(7)	10.7	(9)	0.63	0.79

Table IV. Age and gravida and autoantibody occurrence (N = 136)

		Positive autoantibody tests							
		Thyroid microsome		*Smooth muscle*		*Antinuclear*		*Gastric parietal cell*	
	N	*%*	*(No.)*	*%*	*(No.)*	*%*	*(No.)*	*%*	*(No.)*
Age (yr)									
15-24	35	17.1	(6)	8.6	(3)	2.9	(1)	20.0	(7)
25-29	47	21.3	(10)	6.4	(3)	10.6	(5)	4.3	(2)
30-48	54	16.7	(9)	7.4	(4)	18.5	(10)	13.0	(7)
Rank-sum p value		0.70		0.73		0.024		0.93	
Gravida									
0	12	25.0	(3)	8.3	(1)	0.0	(0)	25.0	(3)
1	37	24.3	(9)	10.8	(4)	8.1	(3)	5.4	(2)
2	44	13.6	(6)	4.6	(2)	13.6	(6)	9.1	(4)
3-6	43	16.3	(7)	7.0	(3)	16.3	(7)	16.3	(7)
Rank-sum p value		0.35		0.56		0.20		0.34	

was drawn. Among these subjects, prior live birth, stillbirth, or miscarriage did not correlate with the detection of autoantibodies (Table V). The association between maternal and fetal complications and the detection of autoantibodies was evaluated in subjects who had ever been pregnant. Maternal and fetal complications that occurred at least five times in pregnant subjects included premature rupture of membranes, chorioamnionitis, hypertension, oxytocin use, and fetal resuscitation. The detection of autoantibodies was not significantly associated with any of these variables.

Comment

Both cellular and humoral immunities may be altered during pregnancy. However, reports concerning changes in the immune system during pregnancy are not in agreement. In general, studies in animals and with human cells in vitro suggest a depression of cellular immune responses to viruses,[6] lymphocyte response to mitogens and allogeneic lymphocytes,[7] homograft survival, and delayed hypersensitivity[8] during pregnancy. The effects of pregnancy on humoral immunity are less clear, but immunoglobulin levels have been reported to be unchanged or slightly increased.[9]

Clinical studies also suggest that pregnancy may affect the immune system. The preponderance of autoimmune disorders in women,[10] the improvement of autoimmune disorders during pregnancy,[11] and the changes noted in autoantibody titers with pregnancy[2] imply that sex steroids or other factors may have a direct effect on the immune system.

Pregnancy may have an effect on autoantibody production, but published data are difficult to interpret. Previous studies on autoantibody detection in pregnancy that examined patients with a documented autoimmune disease describe a variety of changes in autoantibody titer. Autoantibody titers have been reported to increase,[1] decrease,[2] or remain unchanged[3] during pregnancy depending on the autoimmune disorder studied. In patients without an autoimmune disease, testing for autoantibodies has yielded conflicting results. For example, autoantibodies directed against nuclei were reported in one study to increase[5] and in another to be unchanged[4] during pregnancy in healthy women. Similarly, the prevalence of rheumatoid factor measured during pregnancy by radioimmunoassay

Table V. Prior pregnancy outcome and autoantibody occurrence

Prior pregnancy outcomes	*N*	*Positive autoantibody tests*							
		Thyroid microsome		*Smooth muscle*		*Antinuclear*		*Gastric parietal cell*	
		%	*(No.)*	%	*(No.)*	%	*(No.)*	%	*(No.)*
Prior live birth									
No	9	11.1	(1)	0.0	(0)	11.1	(1)	0.0	(0)
Yes	88	15.9	(14)	8.0	(7)	13.6	(12)	13.6	(12)
Rank-sum p value		p = 0.70		p = 0.38		p = 0.83		p = 0.24	
Prior miscarriage or stillbirth									
No	74	17.6	(13)	8.1	(6)	13.5	(10)	10.8	(8)
Yes	23	8.7	(2)	4.4	(1)	13.0	(3)	17.4	(4)
Rank-sum p value		p = 0.30		p = 0.54		p = 0.95		p = 0.40	

was reported to be increased[12] but unchanged when measured by an enzyme-linked immunosorbent assay.[13] It is clear that generalizations concerning the effect of pregnancy on autoantibody production are difficult to make, since autoantibody detection depends on numerous factors, including the sample population tested, frequency and interval of testing, the type of assay procedure, and the number of autoantibodies evaluated.

The purpose of the study was to compare the frequency of autoantibodies in a pregnant population with that in a control group and determine if pregnancy or other historical factors were related to the detection of autoantibodies. In the study, the control group consisted of ovulatory women without major systemic illnesses or medical disorders. Previous studies that reported an increase of autoantibodies in a "normal" pregnant population[3, 5] may have been biased by the lack of an adequate control group. Furthermore, it is unclear in these studies whether endocrine, reproductive, and other medical disorders that may have a direct effect on autoantibody production were assessed.

In the present study antibodies directed against numerous cellular antigens were determined in the pregnant population and a healthy female control group by indirect immunofluorescence and agglutination assays. Since previous studies have not performed a comprehensive survey of autoantibodies during pregnancy, an extensive panel of autoantibodies associated with frequent autoimmune disorders was screened. Most assays in pregnant subjects were performed in the third trimester because in previous studies,[4] it was suggested that the prevalence of autoantibodies and the effects of sex steroids may be greatest at that time.

When the frequency of detection of at least one autoantibody was compared between the pregnant population (40.5%) and the control group (46.2%), no statistically significant difference was noted. The frequency of detection of a single autoantibody class was also compared between the pregnant and control subjects, and no significant association of individual autoantibodies in either study group was noted.

To the best of our knowledge, no study has previously reported the percentage of subjects with positive autoantibodies in pregnant and nonpregnant groups when a large panel of autoantibodies was screened. Although the prevalence of at least one detectable antibody in the sample population may appear high, these percentages may depend on the number of autoantibodies tested and the false-positive rate of each test. If each autoantibody had a false-positive rate of 5%, one could expect that 30% of the subjects would be falsely positive on one or more of seven independent tests (probability of a positive test = $100 \times [1 - 0.95^7]$). More important, the frequency of detecting a single autoantibody, such as antibodies against nuclei, is in agreement with previous studies.[14]

Historical factors in the patient's immediate history and familial history could have a direct effect on the detection of autoantibodies. When past medical history, familial history, age, gravidity, and reproductive history were examined in the control and pregnant groups, no single historical factor was significantly associated with the presence or absence of autoantibodies. A trend in the detection of antibodies against nuclei and age was noted, but was not statistically significant.

The results of the present study do not support suggestions that pregnancy either induces or suppresses the production of autoantibodies in healthy subjects. Because the frequency of several of the autoantibodies in our study population was low, we cannot exclude the possibility that pregnancy may affect the production of these infrequent antibodies. Larger studies will be necessary to determine if an association exists. Since patients in the present study were sampled primarily in the third trimester, we cannot exclude the possibility

that autoantibody production varies with the time of gestation. For example, it has been reported that the detection of antibodies against nuclei may vary throughout pregnancy.[4] A longitudinal study with sampling before pregnancy, during pregnancy, and postpartum could help determine the effect of pregnancy on autoantibody production.

Autoantibodies that react with fetal antigens have been implicated as causes of fetal morbidity in subjects with an autoimmune disorder[1] and may play a role in the pathogenesis of repeated pregnancy loss and pregnancy-induced hypertension.[15, 16] In this study, the presence of the autoantibodies in subjects without a documented autoimmune disease did not appear to have an adverse effect on maternal or fetal well-being. In most cases in both pregnant and nonpregnant subjects, the titer of autoantibodies detected was low. The possibility that high levels of autoantibodies may have an adverse or protective effect on pregnancy or pregnancy outcomes was not determined. It should also be noted that not all antibodies associated with maternal disease or poor reproductive outcome, such as antiphospholipid antibodies or antibodies against extractable nuclear antigens, were evaluated in the present study. The prevalence of these important autoantibodies in uncomplicated pregnancies will require future investigation, and larger studies will be necessary to determine quantitative and qualitative effects of autoantibodies on maternal and fetal outcomes.

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The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly

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We have previously demonstrated that the fetus with anencephaly is hypercholesterolemic. The plasma levels of total cholesterol and low-density lipoprotein cholesterol are threefold greater than those of normal fetuses. We have provided evidence that elevated low-density lipoprotein cholesterol levels were caused by reduced uptake and metabolism of low-density lipoprotein by atrophic adrenal glands deficient in low-density lipoprotein receptors. The purpose of the present investigation was to determine if other tissues, namely, skin fibroblasts, of the fetus with anencephaly were also deficient in low-density lipoprotein receptors. We compared the binding and jmetabolism of low-density lipoprotein by skin fibroblasts of fetuses with anencephaly and normal subjects. Cultures of skin fibroblasts were grown to confluency. Thereafter, the medium was changed to lipoprotein-deficient serum for 24 hours. The rate of uptake and degradation of iodine 125–iodo-LDL was determined as a function of time and concentration of low-density lipoprotein. The maximal specific binding of low-density lipoprotein was also determined. The rate of uptake, degradation, and the maximal binding of low-density lipoprotein was similar in skin fibroblasts of infants with anencephaly and normal subjects. We conclude that the elevated level of low-density lipoprotein cholesterol in cord blood of fetuses with anencephaly is not caused by deficiency of low-density lipoprotein receptors and metabolism in skin fibroblasts but instead by deficiency of low-density lipoprotein receptors and metabolism by atrophic adrenal glands. (Am J Obstet Gynecol 1987;157:1351-5.)

Key words: Low-density lipoprotein, cholesterol, anencephaly

In contrast to normal pregnant women, women with fetuses with anencephaly have very low levels of plasma and urinary estrogens.[1] The low estrogen production by the fetoplacental unit of such pregnancies is the result of reduced production of fetal adrenal C_{19}-steroid precursors, especially dehydroepiandrosterone sulfate.[2, 3] The reason for the reduction in dehydroepiandrosterone sulfate production is that the adrenal glands of fetuses and newborns with anencephaly are atrophic and involuted, presumably because of low levels of circulating adrenocorticotropic hormone compared with those in fetuses with normal neural development.[4, 5]

We have previously demonstrated that the major source of fetal adrenal steroid biosynthesis is cholesterol in the form of low-density lipoprotein.[6] The use of low-density lipoprotein cholesterol involves the binding of low-density lipoprotein to a specific plasma membrane receptor, which is followed by internalization of low-density lipoprotein through a process of adsorptive endocytosis. The internalized vessicles fuse with lysosomes, and the protein component of low-density lipoprotein is hydrolyzed by lysosomal proteolytic enzymes to amino acids. The cholesterol esters of low-density lipoprotein also are hydrolyzed, and the liberated cholesterol is available as a precursor for the biosynthesis of steroid hormones and other cellular processes.[6] We have concluded that the fetal plasma level of cholesterol, and in particular low-density lipoprotein cholesterol, is determined primarily by the rate of uptake and use by the fetal adrenal gland.[2, 3] The anencephalic fetal adrenal gland has a reduced capacity to use low-density lipoprotein because of diminished adrenal low-density lipoprotein receptors.[7, 8] In infants and fetuses with anencephaly, plasma total cholesterol and low-density lipoprotein cholesterol levels are extraordinarily high, whereas dehydroepiandrosterone sulfate levels are extremely low.

In the present investigation, we sought to determine whether hypercholesterolemia, which is caused by excessive levels of low-density lipoprotein cholesterol, in fetuses with anencephaly was attributable solely to deficiencies in the low-density lipoprotein transport apparatus of the atrophic adrenal glands of such fetuses or whether there were also generalized defects in the low-density lipoprotein receptor in other tissues of the fetus with anencephaly. To this end, we investigated

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the low-density lipoprotein receptor system of a representative, well-characterized tissue, namely, skin fibroblasts of normal infants and fetuses with anencephaly.

Material and methods

Skin biopsy specimens were obtained from three fetuses with anencephaly, 20 to 43 weeks' gestation, delivered by spontaneous or induced labor. Tissues were obtained in accordance with the Donors Anatomical Gift Act of the State of Texas after written consent was obtained from the parents with a consent form and protocol approved by the Institutional Review Board of The University of Texas Health Science Center at Dallas. Normal fibroblasts were obtained from the genital skin of two newborn infants undergoing circumcision, donated by Drs. J. D. Wilson and J. E. Griffin (Department of Internal Medicine, University of Texas Health Science Center, Dallas, Texas).

Cells. Fibroblasts were established from explants of skin as described by Griffin et al.[9] Cells were maintained in a humidified atmosphere of carbon dioxide (5%) and air (95%) and in media containing EMEM (Gibco, Chagrin Falls, Ohio), 10% fetal bovine serum (KC Biological, Lenexa, Kan.), 1 mmol/L of HEPES (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), 2% antibiotics, antimycotics (Gibco), and 50 ng × ml^{-1} of fibroblast growth factor (BR Laboratories, Gaithersburg, Md.) until confluency was obtained.

Lipoproteins. Low-density lipoproteins (density, 1.019 to 1.063 gm × ml^{-1}) and lipoprotein-poor serum were prepared as previously described.[10] Iodine 125–iodo-LDL was prepared as previously described.[11] The specific activity of [^{125}I]iodo-LDL ranged from 300 to 500 cpm × ng^{-1} of protein.

Uptake of [^{125}I]iodo-LDL by fibroblasts. Cells were prepared and maintained as previously described. The media used was that described previously for maintaining the fibroblast cultures but fetal bovine serum was deleted and substituted with lipoprotein-poor serum (10% v/v) for 48 hours. Thereafter, the media was replaced with fresh lipoprotein-poor serum media and [^{125}I]iodo-LDL was added in various concentrations (0 to 200 μg × ml^{-1}) for various periods of time as described in the Results section. The incubation was continued at 37° C in a humidified atmosphere of 95% air and 5% carbon dioxide. At the end of the incubation period, the dishes were moved from the incubator and placed on ice. The media was removed, and the cells were rinsed three times with cold 0.15 mol/L of NaCl. The cells were removed with a rubber policeman and homogenized in 1 ml 0.15 mol/L of NaCl; an aliquot (500 μl) was assayed for radioactivity. Duplicate aliquots (100 μl) were assayed for protein content.

Degradation of [^{125}I]iodo-LDL by fibroblasts. To measure the degradation of [^{125}I]iodo-LDL to amino acids (monoiodotyrosine), the culture medium from fibroblasts incubated in the presence of [^{125}I]iodo-LDL was removed from each culture dish and mixed with 1 ml of trichloroacetic acid (50% w/v). Three milliliters of bovine serum albumin (5 mg × ml^{-1}) was added to the acidified culture medium, and the mixture was allowed to stand for 30 minutes at 4° C. The tubes were centrifuged at 1500 rpm for 5 minutes. An aliquot (3 ml) of supernatant was mixed with 30 μl of KI solution (40% w/v) and 120 μl of hydrogen peroxide (30%). After standing at room temperature for 5 minutes, the mixture was extracted with 10 ml of $CHCl_3$ to remove the free iodine, and 2 ml aliquots of the aqueous layer were assayed for radioactivity. We evaluated the extent of nonbiologic degradation of radiolabeled low-density lipoprotein in each experiment. The radioactivity contained in trichloroacetic acid–soluble substance in the culture medium incubated with [^{125}I]iodo-LDL in the absence of cells was assayed. This value served as the blank and was used in the calculation of the results.

Results are expressed as nanograms of [^{125}I]iodo-LDL taken up or degraded per milligram of protein per unit time, and each data point represents the mean ± SE of five replicate dishes.

Binding of [^{125}I]iodo-LDL by fibroblasts. The binding of [^{125}I]iodo-LDL by skin fibroblasts was determined by incubating cells in the presence of [^{125}I]iodo-LDL (200 μg × ml^{-1}) and in the presence or absence of excess nonradiolabeled low-density lipoprotein (2 mg × ml^{-1}) at 4° C to prevent degradation for a period of 6 hours. The maximal specific binding of low-density lipoprotein to skin fibroblasts was determined by substracting the total binding from the nonspecific binding (binding in the presence of an excess of nonradiolabeled low-density lipoprotein).

Chemicals. Bovine serum albumin and trichloroacetic acid were obtained from Sigma Chemical Co. (St. Louis, Missouri). Other chemicals used were of analytic grade.

Statistical analysis of the data was performed by means of an analysis of variance followed by Newman-Keuls multiple comparison analysis.

Results

The uptake and degradation of [^{125}I]iodo-LDL (20 μg × ml^{-1}) by anencephalic and normal skin fibroblasts as a function of time are presented in Fig. 1. The uptake of [^{125}I]iodo-LDL began after it was added to the culture medium, but after 4 hours there was no further net accumulation. During the first hour of incubation, the rate of degradation of [^{125}I]iodo-LDL was low, but

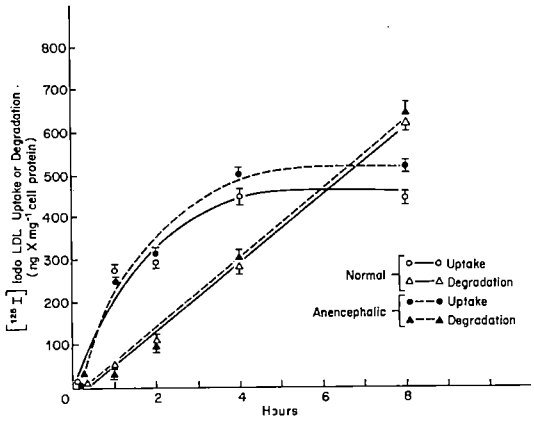


Fig. 1. Time course of uptake and degradation of [^{125}I]iodo-LDL by skin fibroblasts of normal fetuses and those with anencephaly. Cells were preincubated in medium containing lipoprotein-poor serum for 24 hours. At time zero, [^{125}I]iodo-LDL was added to the culture medium to give a final concentration of 20 μg of low-density lipoprotein protein × ml^{-1}). At the times indicated, the uptake and degradation of [^{125}I]iodo-LDL were determined in cells maintained at 37° C. The results are expressed as nanograms per milligram of protein × 6 h^{-1} of uptake or degradation. Each value presented is the mean ± SE (n = 5).

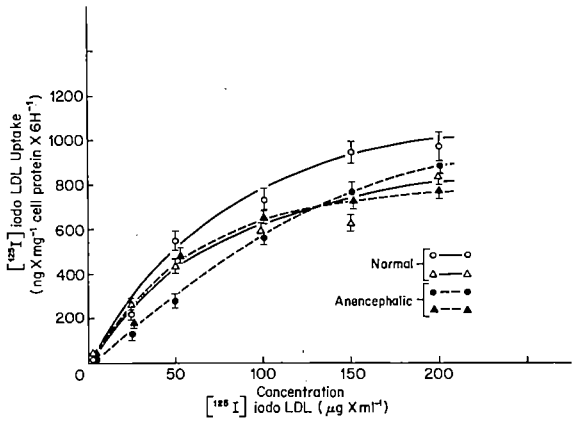


Fig. 2. Uptake of [^{125}I]iodo-LDL by skin fibroblasts of two normal newborns and two fetuses with anencephaly as a function of [^{125}I]iodo-LDL concentration in the culture medium. Cells were incubated in medium containing lipoprotein-poor serum for 24 hours; thereafter [^{125}I]iodo-LDL in various amounts was added and the incubation was continued for 6 hours at 37° C. The rate of uptake of [^{125}I]iodo-LDL was determined as described in the Material and Methods section. Each value represents the mean ± SE (n = 5).

thereafter degradation increased in a linear fashion for at least 8 hours. Based on these results, 6 hours was chosen as the standard time of incubation for further experiments in which time was not a variable. Nonspecific degradation (i.e., degradation of [^{125}I]iodo-LDL in the absence of cells) was extremely low (data not shown). The results of these experiments show that the metabolism of [^{125}I]iodo-LDL as a function of time was similar in anencephalic and normal skin fibroblasts.

The effect of [^{125}I]iodo-LDL in various concentrations (0 to 200 μg of low-density lipoprotein protein × ml^{-1}) on the rate of uptake and degradation of low-density lipoprotein by normal and anencephalic skin fibroblasts is presented in Figs. 2 and 3, respectively. In these experiments, the maximal uptake of [^{125}I]iodo-LDL as a function of [^{125}I]iodo-LDL concentration was similar in anencephalic and skin fibroblasts. Maximal uptake was achieved at concentrations of 150 to 200 μg of [^{125}I]iodo-LDL protein × ml^{-1} (Fig. 2). Likewise, the rate of degradation of [^{125}I]iodo-LDL by anencephalic and normal skin fibroblasts was similar (Fig. 3). The rate of degradation was linear with respect to the concentration of [^{125}I]iodo-LDL.

The maximal specific-binding capacity was determined in anencephalic and normal skin fibroblasts that had been previously maintained for 2 days in medium containing lipoprotein-poor serum. Afterward, fresh medium was added, and the cells were maintained for

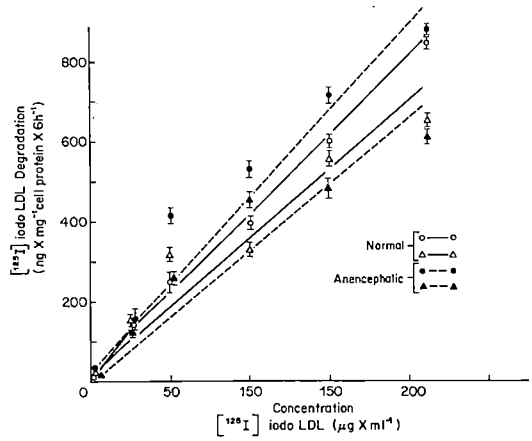


Fig. 3. Degradation of [^{125}I]iodo-LDL by skin fibroblasts of two normal infants and two fetuses with anencephaly as a function of [^{125}I]iodo-LDL concentration in the culture medium. Cells were incubated as described in Fig. 2 at 37° C. The rate of degradation of [^{125}I]iodo-LDL was determined as described in the Material and Methods section. Each value represents the mean ± SE (n = 5).

1 hour at 0° C. Next, [^{125}I]iodo-LDL (200 μg × ml^{-1}) was added in the presence or absence of excess amount of nonradiolabeled low-density lipoprotein (2 mg/ml) for 4 hours. The specific-binding low-density lipoprotein was determined as described in the Material and Methods section and is presented in Fig. 4. The maximal specific binding of low-density lipoprotein was similar in anencephalic and normal skin fibroblasts.

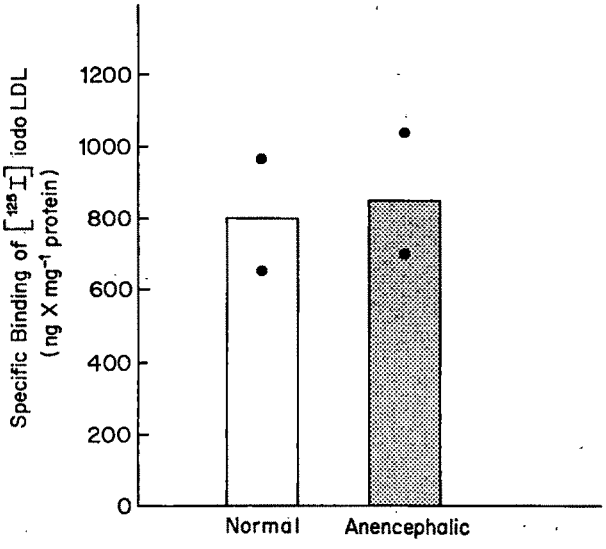


Fig. 4. Comparison of the specific binding capacity for [^{125}I]iodo-LDL of skin fibroblasts from two normal infants and two with anencephaly. The specific binding was determined as described in the Material and Methods section. The results represent the average of two experiments in which there were five replicates.

Comment

The fetus with anencephaly is hypercholesterolemic.[2,3] In fact, the cord levels of total cholesterol and low-density lipoprotein cholesterol are twofold to fourfold greater than the corresponding levels observed in normal infants. We have previously provided evidence to suggest that these differences in total cholesterol and in particular low-density lipoprotein were because of a deficiency in the number of low-density lipoprotein receptors and metabolism of low-density lipoprotein by the atrophic adrenal glands of the fetus with anencephaly.[7,8] The normal human fetal adrenal gland has the highest number of low-density lipoprotein receptors of any human tissue, as well as the tissues of other species.[12] Furthermore, the fetal zone has more low-density lipoprotein receptors than does the neocortex.[13] This partly explains (along with the low levels of adrenocorticotropic hormone), the reduced number of low-density lipoprotein receptors of the anencephalic adrenal gland, which is composed primarily of neocortex tissue.[6] As previously discussed, the uptake and metabolism of low-density lipoprotein by the adrenal gland of normal fetuses to steroid hormones appear to regulate the levels of total and low-density cholesterol in fetal plasma.[2,3]

The results of the present study demonstrate that other tissues, namely, skin fibroblasts of fetuses with anencephaly, contain a similar number of low-density lipoprotein binding sites and rates of metabolism of low-density lipoprotein as do normal skin fibroblasts. In a previous study we demonstrated that the specific activity and amount of 3-hydroxy-3-methylglutaryl coenzyme A reductase (the rate-limiting step in cholesterol biosynthesis) were very low in liver tissue of fetuses with anencephaly compared with that of normal fetuses.[14] This observation suggested that the synthesis of cholesterol in the liver of fetuses with anencephaly was suppressed by the high levels of plasma low-density lipoprotein cholesterol. These studies also suggested that anencephalic liver tissues contained low-density lipoprotein receptors. We have recently observed that anencephalic fetal hepatocytes metabolize low-density lipoprotein at rates similar to that observed for normal hepatocytes (data not shown).

In conclusion, the elevated levels of total cholesterol and low-density lipoprotein cholesterol in cord plasma of fetuses with anencephaly is caused primarily by a deficiency of low-density lipoprotein metabolism and receptors by atrophic adrenal glands. The binding and metabolism of low-density lipoprotein by other tissues, namely, skin fibroblasts and liver of infants with anencephaly and normal fetuses, are similar.

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Intrapartum vibratory acoustic stimulation of the human fetus during episodes of decreased heart rate variability

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The effects of intrapartum vibratory acoustic stimulation during periods of decreased fetal heart rate variability were studied in 25 healthy term fetuses. Fetal monitoring and real-time ultrasound scanning were used simultaneously to detect fetal response. Vibratory acoustic stimulation was provided by an artificial larynx generating a signal at 85 dB and 85 Hz. This stimulus was applied for 5 seconds on the maternal abdomen over the fetal head after a 20-minute period of decreased fetal heart rate variability. All fetuses reacted with an immediate fetal heart rate acceleration of at least 10 bpm (range: 10 to 35 bpm, mean ± SD = 18.4 ± 7.0), and 19 fetuses also had sudden fetal body movement. A deceleration of the fetal heart rate after the initial acceleration was observed in nine fetuses (range: 15 to 70 bpm, mean ± SD = 45.5 ± 16.5). The implications of these findings are discussed in relation to the possible use of fetal vibratory acoustic stimulation for intrapartum surveillance. (Am J Obstet Gynecol 1987;157:1355-8.)

Key words: Healthy fetuses, decreased heart rate variability, vibratory acoustic stimulation

The assessment of fetal heart rate (FHR) variability and its application as a powerful intrapartum indicator of the immediate fetal condition have received increased attention in recent years. Although normal FHR variability is associated with a normal fetal outcome in most cases, decreased fetal heart rate variability commonly presents a clinical dilemma. The differential diagnosis ranges from physiologic fetal sleep to prematurity, congenital anomalies, and central nervous system depression induced by maternal medication or fetal asphyxia.[1] Because maternal medication and prematurity are tangible factors that are easily ruled out, the challenge exists in attributing decreased fetal heart rate variability to either quiet fetal sleep or fetal asphyxia.

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Studies have shown that the fetus is responsive to external stimuli such as maternal glucose administration, light, sound, vibration, and physical shaking of the maternal abdomen.[2-5] These studies examined the FHR changes in response to external stimuli applied during the antepartum period. Recently it has been shown that antepartum vibratory acoustic stimulation is capable of eliciting consistent fetal responses. Healthy term fetuses responded to this stimulus by a sudden body motion (the "startle reflex") and notable heart rate acceleration.[2, 6-8]

The purpose of this study was to document fetal response to vibratory acoustic stimulus as measured by FHR and fetal movement during the active phase of labor and in the presence of decreased FHR variability.

Material and methods

Twenty-five healthy pregnant women at full-term gestation and in active labor were included in the study. Active labor was defined as the onset of strong regular uterine contractions within intervals of 5 minutes or less and a cervical dilation of at least 4 cm. No patients

Table I. Immediate FHR increase in response to vibratory acoustic stimulation

Patient No.	*Increase in FHR (bpm)*	*Duration (sec)*	*Fetal movement*
1	20	130	+
2	25	120	+
3	25	10	+
4	10	10	+
5	25	10	−
6	10	15	+
7	15	60	−
8	30	150	+
9	10	10	+
10	10	10	+
11	15	45	−
12	10	5	+
13	20	10	+
14	20	10	+
15	15	5	+
16	20	30	+
17	15	30	+
18	25	140	+
19	20	20	+
20	35	90	−
21	15	25	−
22	15	5	+
23	30	20	+
24	10	5	+
25	15	120	−
	Mean ± SD = 18.4 ± 7.0 Range, 10-35	Mean ± SD = 43.4 ± 48.4 Range, 5-150	

were medicated. All fetuses were in cephalic presentation. Only uncomplicated singleton pregnancies with normal uterine contraction–FHR patterns were studied.

A 20-minute period of decreased FHR variability (i.e., amplitude range <6 bpm) was recorded before application of the vibratory acoustic stimulus. FHR was monitored with a fetal monitor (Corometrics 115, Corometrics Inc., Wallingford, Conn.). Fetal movement was documented by ultrasonography with a real-time scanner (Ultramark IV, ATL, linear array transducer, 3.5 MHz, Tempe, Ariz.), with the transducer placed longitudinally over the fetal chest and upper abdomen.

The fetus was observed for approximately 1 minute, and in the event of no detected fetal movement, a single 5-second vibratory acoustic stimulus was applied on the maternal abdomen over the fetal head. The vibratory acoustic stimulus measured at approximately 85 dB and 85 Hz (measured in air at a distance of 1 m) was generated by an artificial larynx (Model 5C, Western Electric). Fetal movement was defined as a sudden movement of the fetal body (i.e., startle reflex) occurring during the stimulus interval or within 2 seconds of its cessation.[7] The same examiner conducted all tests.

Results

All fetuses delivered in this study were deemed healthy on the basis of Apgar scores and physical examination. No newborn infant had an Apgar score of less than 8 at 5 minutes. All fetuses were appropriately grown and had an adequate amount of amniotic fluid. Every fetus uniformly showed an initial FHR increase during or immediately after the vibratory acoustic stimulus of at least 10 bpm. The increase in FHR measured 18.4 ± 7.0 (mean ± SD) bpm for a duration of 43.4 ± 48.4 seconds. The range for FHR increase was 10 to 35 bpm and its duration spanned from 5 to 150 seconds (Table I and Fig. 1, *A*).

In nine fetuses the initial FHR increase was followed by a FHR deceleration of 45.5 ± 16.5 bpm (mean ± SD) for 25.5 ± 19.2 seconds. The range of FHR decrease was 15 to 70 bpm for a duration of 10 to 60 seconds (Fig., 1, *B*). Three of these nine fetuses had associated umbilical cord problems.

Fetal movement occurring during the stimulus or within 2 seconds of its cessation was observed in 19 of 25 patients (Table I). No fetal movement was detected during the 1-minute observation period before stimulus administration.

In all patients fetal movement was associated with an increase in FHR. In six patients, however, the increase in fetal heart rate was not associated with simultaneous fetal movement. There was also no correlation between the increase and duration of FHR changes and the presence of fetal movement. Among the 12 fetuses who responded with an acceleration of more than 15 bpm and 15-second duration, five had no fetal movement. Only one of the 13 fetuses who responded to the stim-

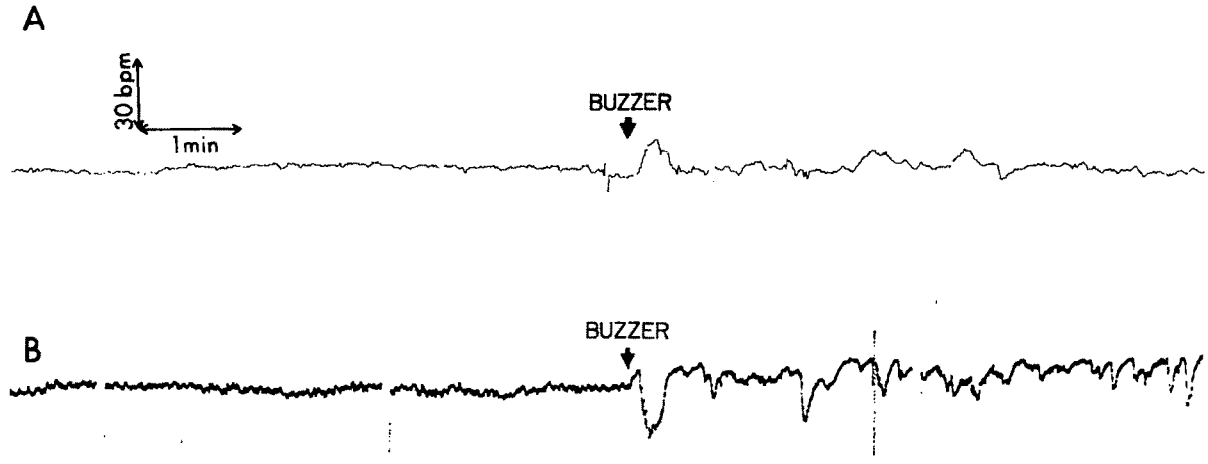


Fig. 1. A, A typical acceleration of the FHR in response to vibratory acoustic stimulation. **B,** A typical FHR deceleration after an initial acceleration in response to vibratory acoustic stimulation.

ulus with a FHR acceleration of 10 to 15 bpm had no fetal movement.

Comment

The results of this study showed that intrapartum vibratory acoustic stimulation of healthy term fetuses during episodes of decreased FHR variability evoked an immediate FHR acceleration of at least 10 bpm. These results were demonstrated in all fetuses tested. Nine of the 25 fetuses demonstrated a FHR deceleration after the initial acceleration. Fetal movement in response to this stimulus was less consistent than the FHR response but was still elicited in 19 of the 25 study patients.

Recent studies have shown that human fetal activity is strongly correlated with behavioral states. Several studies identified recurrent cycles of normal and decreased FHR variability in healthy near term pregnancies.[1] Similarly, consecutive episodes of reduced and normal FHR variability consistent with cycles of quiet and active behavior were found in active labor.[9]

All fetuses were stimulated after a 20-minute period of decreased FHR variability and demonstrated an immediate change in FHR and fetal movement in response to vibratory acoustic stimulation. Spencer et al. studied healthy term fetuses in active labor and showed that the mean duration of episodes of decreased FHR variability was 25 minutes. Since all fetuses evaluated in the present study were healthy by outcome criteria, it is likely that the decrease in FHR variability was associated with a fetal sleep state and that our observations recorded the response of the healthy "sleeping" fetus to vibratory acoustic stimulus.

FHR and fetal movement were the two parameters used to evaluate fetal response to vibratory acoustic stimulus in this study. In an antepartum study, Leader et al.[2] showed that FHR and fetal movement are variables that respond independent of each other. In the present study, no correlation existed between changes in FHR and the presence of fetal movement. However, our ability to assess the value of fetal movement was limited because no fetus responded to vibratory acoustic stimulation by fetal movement alone. Six fetuses responded to the stimulation by FHR acceleration with no concomitant fetal movement. It is, of course, possible that fetal movement occurred in alternative planes to the one observed by the real-time ultrasound scanner. It is also possible that the increase in FHR was caused by a fetal stress reaction with a concomitant increase in sympathetic activity and no fetal movement.[11]

The presence of spontaneous FHR accelerations during labor is well correlated with positive fetal outcome; yet, their absence is poorly associated with fetal distress.[11,12] Several studies suggest the use of external fetal stimulation as a way to evoke FHR accelerations. However, it has yet to be determined whether such evoked accelerations are as predictive of good fetal outcome as are spontaneous accelerations. In the study of Clark et al., all fetuses who responded to scalp stimulation with a FHR acceleration were vigorous at birth. Smith et al.[6] used vibratory acoustic stimulation of the fetus and showed that the evoked reactive nonstress test was as predictive of fetal health as the spontaneous nonstress test.

In the present study, all fetuses responded with an immediate FHR acceleration and fetal outcome was uniformly good. A possible explanation for this increase in FHR may be provided by partial occlusion of the umbilical vein caused by fetal movement. This occlusive event may precipitate a decrease in fetal blood pressure and acute hypoxia that results in reflex stim-

ulation of the autonomic nervous system. If complete occlusion occurs in the fetus without acidosis, the initial FHR acceleration is likely followed by a cardiac deceleration.[14] Total occlusion may occur when an obvious umbilical cord problem is present. In the present study, the nine decelerations observed caused by the stimulus were preceded by an increase in the FHR. Three of these patients had a clear cord problem on delivery. As expected, none of these fetuses was asphyxiated.

Ohel et al.[15] used intrapartum vibratory acoustic stimulation in cases of normal and abnormal FHR patterns. Their results indicate a significantly greater incidence of acceleration deceleration in FHR responses in fetuses with abnormal FHR tracings compared with fetuses with normal FHR tracings. Immediate FHR deceleration or zero response triggered by external stimulation was related to poor fetal outcome by Read et al.[3] Likewise, Luz et al.[16] suggested that a fetus may lose its ability to respond to an external sound or vibration stimulus before other signs of fetal distress are present. The possibility of generating a cardiac deceleration by evoking fetal responses and using this information toward differentiating umbilical cord compression from placental insufficiency is intriguing and warrants a prospective evaluation.

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Postprandial walking exercise in pregnant insulin-dependent (type I) diabetic women: Reduction of plasma lipid levels but absence of a significant effect on glycemic control

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In this study 42 pregnant women with type I diabetes and 28 nondiabetic controls were recruited to participate in a postprandial walking exercise program. Exercise patients were instructed to walk 20 minutes (1 mile) after each meal and were divided into two groups: group 1 were normal nondiabetic controls and group 2 were women with type I diabetes. There were two nonexercise comparison groups: group 3, nondiabetic controls, and group 4, women with type I diabetes. Diabetic women were followed weekly in an intensive perinatal program. Glycemic control was assessed by serial hemoglobin A_1 concentration measurements, home blood glucose monitoring, 24-hour glucose profiles, and 24-hour quantitative urinary glucose loss. Glycemic control was modestly but not significantly superior in the diabetic exercise group 2 compared with the diabetic nonexercise group 4. Exercise was associated with lower fasting cholesterol and triglyceride values in both controls and diabetic women, with significantly lower fasting plasma triglyceride levels in the diabetic exercise group ($p < 0.02$). There were no adverse effects of postprandial walking exercise in mothers or infants. (Am J Obstet Gynecol 1987;157:1359-63.)

Key words: Pregnancy, type I diabetes, exercise, triglyceride

In pregnant insulin-dependent (type I) diabetic women, exercise has not been a standard therapeutic recommendation to accompany insulin administration and dietary recommendations. Moreover, the American College of Obstetricians and Gynecologists[1] has defined a group of risk factors, including diabetes, as relative contraindications for vigorous physical activity during gestation. In contrast, moderate exercise is a usual recommendation for normal pregnant women, and guidelines for various types of physical activity have been proposed.[1, 2]

Several groups of investigators[3-8] have published optimistic reports of the effect of short-term exercise on glucose control in nonpregnant individuals with type I diabetes. Others, however, have failed to document a beneficial effect of physical exercise or training on glycemic control in individuals who have inadequate pancreatic β cell secretion of insulin.[9-11] Moreover, adverse metabolic and hormonal effects occur with exercise in diabetic patients who are in poor control.[12]

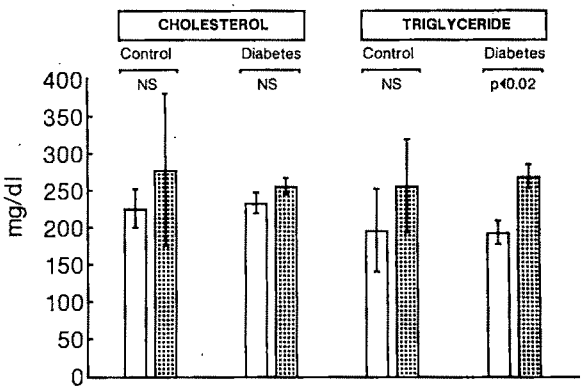


Fig. 1. Fasting plasma cholesterol and triglyceride levels in pregnant normal and type I diabetic women at 35 to 38 weeks' gestation. Exercise patients are represented by the *open bars* and nonexercise patients are represented by the *stippled bars*. Triglyceride values were significantly lower in diabetic women who exercised ($p < 0.02$). The *bars* represent the standard error of the mean.

Many young insulin-dependent diabetic women are actively engaged in sports programs and recreational forms of exercise. They frequently wish to continue these activities during pregnancy. At present, there are no published reports of the effects of prolonged mild,

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Table I. Walking exercise in pregnant women with insulin-dependent diabetes (type 1) and normal controls

Patients	*No.*	*Age (yr)*	*Prepregnant weight (kg)*	*Weight gain (kg)*	*Exercise (miles/day)*	*Length of gestation (wk)*
Exercise						
Group 1	12	26 ± 5	58 ± 9	15 ± 4	6.3 ± 2*	40 ± 1.5†
Group 2	13	25 ± 4	57 ± 6	15 ± 3	4 ± 1‡	37 ± 2.4
No exercise						
Group 3	8	24 ± 4	57 ± 6	15 ± 3	3.0 ± 0.1	39.7 ± 1.2†
Group 4	21	25 ± 4	59 ± 10	16 ± 6	2.2 ± 0.5	37 ± 2.0

Data are mean ± SD.
*$p < 0.01$.
†$p < 0.001$.
‡$p < 0.003$.

moderate, or endurance types of exercise throughout pregnancy on trained, untrained, or physically fit or unfit diabetic women without residual β-cell function.

Our purpose was to assess the effect of postprandial walking exercise in pregnant, untrained, normal, and diabetic women and their infants.

Material and methods

Subjects. Forty-two women with insulin-dependent (type I) diabetes (fasting plasma C-peptide levels <1.0 ng/dl) were recruited from the Diabetes in Pregnancy Clinic at the University of California, San Diego Medical Center (UCSD) and randomly assigned to a walking exercise protocol or control group. A comparison group of 28 normal pregnant women were referred from UCSD primary obstetric care clinics and private obstetricians. All subjects were of normal weight and similar chronologic and gestational ages. Informed consent was obtained from each participant.

Protocol. All subjects were admitted to the General Clinical Research Center (GCRC) in the late first or early second trimester, serially at 21 to 27 and 35 to 38 weeks' gestation, to assess diabetic control as indicated by hemoglobin A_1 concentrations,[13] mean 24-hour glucose values, 24-hour quantitative urinary loss of glucose, and fasting plasma lipid levels[14] as previously described.[15] During the baseline study period, each diabetic patient received instructions in a diabetic diet[16] and each normal pregnant subject was placed on the diet recommended for pregnancy.[17]

Exercise and nonexercise patients were given walking pedometers that were appropriately calibrated to their strides. Subjects in the exercise protocol (group 1, normal women; group 2, women with type I diabetes) were instructed to walk 20 minutes after each meal and to record the pedometer reading for each period and at bedtime. Controls in the nonexercise groups (group 3, normal women; group 4, women with type I diabetes) were given no activity instructions, but requested to record the pedometer reading each night at bedtime. All patients kept daily compliance records for both exercise and dietary intake, adapted from our previously published method for dietary compliance.[18] Information recorded on the compliance forms concerning extraneous activity and nocturnal pedometer readings permitted an assessment of activity among and between groups.

All diabetic women were followed weekly in the UCSD Diabetes in Pregnancy Clinic for obstetric care, to review results of home blood glucose monitoring (four times daily) and for adjustments in insulin doses and diet. We assessed the daily exercise and dietary compliance forms at each visit.[18] Normal pregnant women were followed by their primary physicians and they brought or mailed their exercise and dietary records to the GCRC each week. Data were analyzed by Student's *t* test and group *t* tests. Random variation is reported as ±1 SD or SEM as indicated. All p values are two tailed.

Results

Observations. In the diabetic exercise group (group 2), one subject had a spontaneous abortion before walking exercise was initiated and four withdrew during the course of the study. Among diabetic controls (group 4), there were two spontaneous abortions before normal activity was monitored and one patient dropped out. In the nondiabetic comparison, groups 1 and 3, four women in each group withdrew from the study.

The mean (±SD) gestational age on entry into the program was 12.7 ± 7 weeks for diabetic exercise group 2, versus 12.3 ± 3 weeks for diabetic control group 4. The duration of study was 24 ± 4 weeks for group 2 and 22 ± 6 weeks for group 4. Nondiabetic controls were followed for a similar period. As shown in Table I, there were no differences among the four groups in age, prepregnancy weight, or weight gain during gestation. Length of gestation was shorter in diabetic patients than in nondiabetic controls ($p < 0.001$). The range of gestational ages in diabetic

Table II. Effect of walking exercise on diabetic control and insulin requirements during pregnancy

Patients	No.	*Hemoglobin A_1 (%)*		*Mean 24 hr blood glucose (mg/dl)*		*Mean 24 hr urine glucose (gm)*		*Total insulin dose (U/24 hr)*	
		Baseline	*End*	*Baseline*	*End*	*Baseline*	*End*	*Baseline*	*End*
Exercise									
Group 1	12	NS*		NS		NS			
		5.6 ± 0.5	5.6 ± 0.4	89 ± 6	88 ± 9	0.4 ± 0.3	0.5 ± 0.4	0	0
Group 2	13	$p < 0.001$		$p < 0.001$		$p < 0.001$			
		11.8 ± 2.5	7.8 ± 1.4	174 ± 59	103 ± 36	36 ± 30	4 ± 6	44 ± 20	92 ± 36
No exercise									
Group 3	8	NS*		NS		NS			
		6.1 ± 0.7	6.1 ± 0.5	86 ± 5	85 ± 7	1.2 ± 2	0.7 ± 7	0	0
Group 4	21	$p < 0.001$		$p < 0.001$		$p < 0.001$			
		10.5 ± 3.4	8.3 ± 1.5	165 ± 55	111 ± 33	44 ± 43	8 ± 13	46 ± 16	98 ± 30

Data are mean ± SD.

*Values were lower in exercising, nondiabetic controls (group 1) than in nondiabetic, nonexercise controls (group 3; $p < 0.05$).

Table III. Maternal complications in normal and type I diabetic women who exercised during pregnancy

Patients	No.	*Pregnancy-induced hypertension*	*Preterm labor*	*Premature rupture of membranes*	*Polyhydramnios*	*Pyelonephritis*	*Chorioamnionitis*	*Endometritis*
Exercise								
Group 1	12	0 (0%)	3 (25%)	1 (8%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)
Group 2	13	2 (15%)	2 (15%)	1 (7%)	0 (0%)	0 (0%)	1 (7%)	1 (7%)
No exercise								
Group 3	8	0 (0%)	1 (12%)	0 (0%)	0 (0%)	0 (0%)	1 (12%)	0 (0%)
Group 4	21	2 (9.5%)	0 (0%)	4 (19%)	2 (10%)	1 (5%)	3 (14%)	3 (14%)

women at delivery was 33 to 38.5 weeks in the exercise group and 34 to 40 weeks in the nonexercise group (not significant). The lower gestational age in diabetic versus nondiabetic groups was a reflection of early delivery for pregnancy-induced hypertension, fetal distress, and failed inductions of labor in earlier elective delivery.

Normal women who exercised (group 1) were highly motivated; 11 of 12 subjects exceeded the exercise protocol and eight (66%) walked more than 5 miles a day. This was significantly more walking exercise than the amount performed by the group 3 nondiabetic, nonwalking controls ($p < 0.01$) or women with type I diabetes who exercised (group 2), who in turn walked appreciably more than diabetic women who received no walking instructions (group 4; $p < 0.003$).

Diabetic control and insulin requirements. Table II presents an assessment of diabetic control with measurements of three variables: hemoglobin A_1 concentrations, mean 24-hour blood glucose values, and 24-hour urinary loss of glucose on entry and at completion of the study.

In both diabetic groups there was a striking and significant improvement in glycemic control. The change from baseline to the end of study was somewhat better in the exercise group, but the differences failed to reach statistical significance ($p < 0.06$).

Total insulin doses at baseline and at end of study were almost identical in groups 2 and 4 (Table II). Women in diabetic exercise group 2 did not require less insulin or have a smaller increase in their insulin dose during pregnancy compared with nonexercising diabetic subjects (group 4).

Fasting plasma cholesterol and triglyceride levels. The most interesting and potentially important finding in this study was the observation of lower fasting plasma cholesterol and triglyceride values in both normal and diabetic exercise groups (Fig. 1). In type I diabetic exercise patients (group 2), mean (±SEM) fasting plasma triglyceride levels were significantly lower than those in nonexercising diabetic controls (196 ± 16 versus 269 ± 23 mg/dl; $p < 0.02$). This was in contrast to the similar levels obtained at baseline (109 ± 67 versus 116 ± 39 mg/dl, not significant).

Maternal outcome. In this relatively small study there were no statistically significant differences in maternal complications of pregnancy in diabetic or comparison groups (Table III). We did observe, however, more episodes of preterm labor (20%) among the 25 women who exercised than in the controls (3.4%). Eleven of 12 normal exercise controls (including the three with preterm labor) exceeded the exercise protocol (range 2.9 to 10.4 miles/day). In the diabetic exercise group (range 3.0 to 6.3 miles/day), the three subjects with

Table IV. Complications in newborn infants of type I diabetic women who exercised and normal controls

Patients	No.	*Preterm birth*		*Fetal distress*		*Hypoglycemia*		*Hypocalcemia*		*Hyperbilirubinemia*		*Macrosomia*	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Exercise													
Group 1	12	0	0	0	0	0	0	0	0	1	8	1	8
Group 2	13	4	38	3	23	2	15	0	0	3	23	1	8
No exercise													
Group 3	8	0	8	0	0	1	9	0	0	2	18	0	0
Group 4	21	8	38	2	10	7	33	3	14	10	48	4	19

*Caudal regression syndrome.

preterm labor walked a mean of 4.8, 4.1, and 6.3 miles/day and were delivered in labor at 33, 34, and 35 weeks' gestation.

In our tightly controlled pregnant diabetic women, hypoglycemia, usually mild, occurred in both groups but was not increased in women who walked.

The cesarean section rate was high in both diabetic groups (group 2, seven [54%] and group 4, 17 [81%]). In the diabetic exercise group, cesarean section was performed for fetal distress in three patients, for failure to progress in labor in two, and for malpresentation in two others. In the nonexercise group, three women had a repeat cesarean section, four failed to progress in labor, three had cephalopelvic disproportion, and in two there was failed induction of labor. In the remaining cases, pregnancy was complicated by hypertension (two patients), breech presentation (one), or fetal distress (two). One normal pregnant woman who exercised had a cesarean section for failure to progress in labor.

Outcome of infants. Diabetic exercise subjects (group 2) had smaller placentas (509 ± 128 versus 552 ± 115 gm [$\bar{x}$ ± SD]; not significant) and infants with lower birthweights (3140 ± 658 versus 3582 ± 819 gm; not significant) and lengths (48.7 ± 2.7 versus 51.1 ± 3.6 cm; not significant). There was no difference in body mass index (infant birth weight/length^3) or placental index (placental weight/infant birth weight, or placental weight/body mass index) in the infants of the two groups.

In the 20 normal nondiabetic women there was no discernible effect of walking exercise on placental weight, gestational age, infant birthweight, length, or head circumference.

Table IV shows the complications in all four groups of infants. Diabetic women who exercised had fewer infants with neonatal hypoglycemia, hypocalcemia, hyperbilirubinemia, or macrosomia. One infant had a minor malformation. In the diabetic control group there were minor malformations in two infants and major malformations, one fatal, in two others.

Comment

This is the first study to examine the efficacy of measured walking exercise throughout pregnancy in untrained women without residual pancreatic β-cell function and in normal nondiabetic controls. Walking exercise was selected because it requires no expense or special equipment and can be engaged in by all pregnant women who do not have specific obstetric or diabetic contraindications.[19]

Our study was performed in an ambulatory high-risk obstetrics clinic that uses an intensive team approach, home blood glucose monitoring, weekly clinic visits for assessment of 24-hour urinary loss of glucose, and assessment of dietary and exercise records. In this setting we found somewhat better control of diabetes in type I diabetic women who exercised. However, analysis of end point quantitative measurements and change values failed to demonstrate a significant difference in glycemic control between diabetic groups.

Our results support the experience of others[10, 20-22] who have reported no objective evidence to support the beneficial effect of exercise or physical training on glycemic control in type I (insulin-dependent) nonpregnant diabetic patients. Exercise appears to be effective in normalizing glucose tolerance only in patients who still have an adequate capacity to secrete insulin and in whom insulin resistance is the major cause for abnormal glucose tolerance.[11] Thus it is perhaps reasonable to suggest that exercise might be more beneficial in pregnant women with type II diabetes (noninsulin dependent, but "insulin requiring" during pregnancy), as suggested by Artal and Wiswell,[23] and in obese subjects with gestational carbohydrate intolerance (gestational diabetes, GDM) and hyperinsulinemia.[24]

The observation of an apparent increase in preterm labor in the exercise groups should alert others who are engaged in studies of exercise in pregnancy to this possibility. Additional data concerning this complication are needed in a larger group of subjects.

Are the lower lipid levels in pregnant diabetic women who exercise important? Wallberg-Henriksson et al.[10]

Malformations		*Neonatal death*	
n	%	*n*	%
0	0	0	0
1	8	0	0
0	0	0	0
4	19	1*	5

have reported a modest decrease in total cholesterol and low-density lipoprotein triglyceride levels with daily exercise training. During pregnancy, marked endogenous hypertriglyceridemia occurs.[21, 25] Our exercise study showed significantly decreased plasma triglyceride values in the diabetic exercise group ($p < 0.02$) and lower fasting cholesterol and triglyceride levels in both diabetic and nondiabetic exercise subjects. One might speculate that lower lipid levels altered the nutrient supply transported to the fetus to account for the somewhat smaller babies of comparable gestational ages in the diabetic exercise group. The effect of exercise on the umbilical dietary supply in normal and diabetic pregnancies has not been tested by clinical investigations in humans.

In conclusion, in an intensive perinatal diabetes program, postprandial walking exercise did not significantly improve glycemic control in women with type I diabetes. In diabetic women, exercise was associated with significantly lower fasting plasma triglyceride levels and no increase in complications in mothers or infants.

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Restriction fragment length polymorphisms of the insulin gene hypervariable region in gestational onset diabetes mellitus

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Restriction fragment length polymorphisms in the insulin gene hypervariable region are compared among 93 women with gestational onset diabetes mellitus and 146 women with normal glucose tolerance during pregnancy. No significant differences in gene or genotype frequencies were observed in the overall sample ($p > 0.50$). However, an increased frequency of one allele (class 1) was observed among nonoverweight patients with gestational onset diabetes mellitus with elevated fasting plasma glucose levels compared with age-, race-, and parity-matched control subjects ($p = 0.061$). These data suggest that gestational onset diabetes mellitus is a heterogeneous disorder with respect to both genotypic and phenotypic characteristics, and that restriction fragment length polymorphisms near the insulin gene may serve as a molecular marker for susceptibility to gestational onset diabetes mellitus only in some women. (AM J OBSTET GYNECOL 1987;157:1364-8.)

Key words: Gestational diabetes, insulin gene, restriction fragment length polymorphism analysis, genetic heterogeneity

Diabetes mellitus is a heterogeneous group of disorders that share abnormal glucose tolerance as a common feature. Because the molecular bases of these disorders are unknown, they are generally divided into two major subgroups based on insulin requirements. Insulin-dependent (type 1) diabetes mellitus is characterized by absence of insulin production, associations with other autoimmune disorders, the presence of islet cell antibodies, and associations with human leukocyte antigens HLA-DR3 and HLA-DR4. Thus type 1 diabetes mellitus is thought to have an immunologic pathogenesis in genetically susceptible individuals. Noninsulin-dependent (type 2) diabetes mellitus is characterized by inadequate insulin production, resistance to insulin action, or both. Type 2 diabetes mellitus is not associated with human leukocyte antigens, although twin and family studies suggest that it has a genetic etiology. Genes conferring susceptibility to type 2 diabetes mellitus have not been identified.

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Gestational onset diabetes mellitus, defined as "glucose intolerance of varying severity with onset or first recognition during pregnancy,"[1] is generally considered to be a variant of type II diabetes mellitus. However, it is becoming increasingly evident that gestational onset diabetes mellitus is a heterogeneous disorder with regard to clinical and phenotypic characteristics as well as progression to overt diabetes mellitus under nongravid conditions.[2-5] Recent finding of HLA-DR3 and HLA-DR4 associations and islet cell antibodies in some, but not all, cases of gestational onset diabetes mellitus[2, 3] suggest that some cases may represent a slowly evolving form of type 1 diabetes mellitus. Thus the pathogenesis and underlying genetic susceptibility to gestational onset diabetes mellitus may also be heterogeneous.

The genes conferring susceptibility to gestational onset diabetes mellitus are unknown. However, several genes that either directly or indirectly regulate carbohydrate metabolism have been isolated and are thus amenable for study. These include the insulin,[6, 7] insulin receptor,[8] and glucose transporter[9] genes. It is possible that variants of any of these candidate genes could contribute to the glucose intolerance of gestational onset diabetes mellitus. Our strategy has been to look for a restriction fragment length polymorphism that is a molecular marker for a diabetogenic gene, if it exists. Similar approaches have been used to identify deoxyribonucleic acid (DNA) markers for other human genetic disorders, including single gene defects such as Huntingtons disease[10] as well as polygenic disorders such as atherosclerosis.[11] In our initial studies we examined the

role of the insulin gene in the etiology of gestational onset diabetes mellitus.

The human insulin gene has been cloned, sequenced, and localized to the short arm of chromosome 11 (11p15).[6, 7, 12] A region containing variable numbers of tandomly repeated oligonucleotides has been identified in the 5′-flanking region, about 365 base pairs upstream from the initiation site of insulin transcription.[6] This hypervariable region has been grouped into three size classes, or alleles, designated as class 1, class 2, and class 3. The class sizes correspond to approximately 570, 1320, and 2470 base pairs, respectively. The smallest, class 1 allele is the most common allele in all populations studied. The intermediate size, class 2 allele is rare in whites but occurs in low frequencies (about 10%) in blacks in the United States. The largest, class 3 allele is common in whites and blacks in the United States, but rare in Asians of Japanese and Chinese descent.[13]

Because the structure of DNA in the 5′-flanking region of many genes plays a role in modulating rates of transcription, the relationship between the insulin gene hypervariable region and types 1 and 2 diabetes mellitus has been explored. Initial studies showed an association between the class 3 allele and type 2 diabetes mellitus,[14-16] but later studies failed to confirm these findings.[17, 18] Rather, recent surveys have revealed significant associations between the class 1 allele and types 1 and 2 diabetes mellitus in Caucasians (reviewed in reference 13). Based on pooled data from five studies, the frequencies of the class 1 allele in type 1 diabetes mellitus, type 2 diabetes mellitus, and nondiabetics were 0.87, 0.75, and 0.70, respectively.[13] Based on these data, Bell et al.[13] estimated that the class 1 allele confers a relative risk of 1.3 for type 2 and of 3.3 for type 1 diabetes mellitus. The latter is comparable to the risk associated with HLA-B8. The physiologic or genetic bases for these associations are unknown.

The relationship between the insulin gene hypervariable region and gestational onset diabetes mellitus has not previously been studied.

Material and methods

Study population. All subjects were derived from patient populations at Prentice Women's Hospital and Maternity Center of Northwestern Memorial Hospital and Northwestern University Medical School. All women attending Prentice Ambulatory Care Obstetric Clinics are routinely screened for gestational onset diabetes mellitus between 24 and 28 weeks' gestation. Glucose tolerance testing is offered to all women with glucose values >129 mg/dl 1 hour after a 50 gm glucose load. Diagnosis of gestational onset diabetes mellitus is made on the basis of oral glucose tolerance testing according to the criteria of O'Sullivan and Mahan.[19]

Women diagnosed with gestational onset diabetes mellitus through Prentice Ambulatory Care screening programs were enrolled for study. Control subjects were ascertained from among women with glucose values <130 mg/dl on the routine screen or with all four normal points on the glucose tolerance test. One to two control subjects were selected for each subject with gestational onset diabetes mellitus, matched for age (±2 years), ethnic group (black, white, hispanic), and gravidity (primigravid, multigravid).

All patients with gestational onset diabetes mellitus and the control subjects were interviewed by one of two investigators (C. O., C. W.), during which time personal and family history information were ascertained (including prepregnancy weight, height, birth weight of subject, birth weight of index pregnancy, family history of diabetes mellitus, reproductive history, oral contraceptive use, and cigarette use). One blood sample for DNA analysis was collected from each participant.

DNA analysis. Genomic DNA was prepared from 15 ml of EDTA-treated whole blood as described[6] and stored in solution at 5° C. DNA samples were digested to completion with restriction endonuclease *Rsa* I following manufacturer's (Boehringer Manheim Biologicals, Indianapolis, Indiana) directions, separated by electrophoresis on 1% agarose, and transferred to nitrocellulose.[20] The cloned probe ph*ins*96 (kindly provided by Dr. Graeme Bell, Howard Hughes Institute, University of Chicago, Chicago, Illinois) was ^{32}P labeled by nick translation[21] and hybridized to nitrocellulose-bound DNA at 42° C with 50% formamide, 5X SSC (0.75 mol/L NaCl, 0.075 mol/L NaCi), 10% dextran sulfate, 20 mmol/L sodium phosphate, 2X Denhardt's solution, 0.1% sodium dodecyl sulfate, and 100 μg/ml salmon testes DNA for 48 hours. Filters were washed at 37° and 65° C, each for 1 hour in 0.1% sodium dodecyl sulfate and 0.1X SSC. Hybridized blots were exposed to XRP-1 x-ray film (Kodak) with intensifying screens (Dupont Lightening Plus) at −80° C for 5 days.

An autoradiograph of *Rsa* I–digested DNA hybridized to ph*ins*96 is shown in Fig. 1.

Statistical analysis. Associations between genotypes at the insulin gene hypervariable region and gestational onset diabetes mellitus were measured by the Maentel-Haenzel χ^2 test, stratified by racial group.[22] Allele frequencies between patients with gestational onset diabetes mellitus and control subjects were compared by a z test. To elucidate the potential confounding effects of phenotypic heterogeneity in gestational onset diabetes mellitus, subjects were further stratified on the basis of fasting plasma glucose value on the oral glucose tolerance test[23] and body mass. Patients with gestational onset diabetes mellitus with fasting plasma glucose values below the upper limits of normal for pregnancy (105 mg/dl)[19] were analyzed separately. A body mass index was calculated as: prepregnancy weight

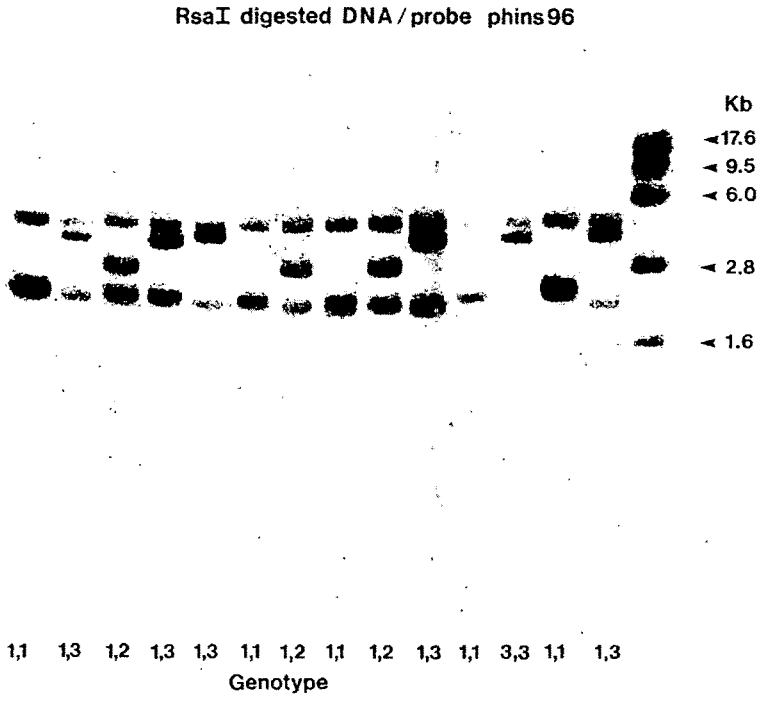


Fig. 1. Autoradiogram of *Rsa* I–digested DNA hybridized to ^{32}P-labeled ph*ins*96. The class 1 allele appears as an approximately 2 kb band and the class 3 allele as an approximately 3.8 kb band; the class 2 allele is of intermediate size. All individuals have a common 4 kb band.

Table I. Sample composition and characteristics

	Size (N)	*Mean age (yr)*	*Mean No. of pregnancies*	*First-degree relative with DM (%)*
Black				
GDM	27	31	3	54
Control	48	28	3	28
White				
GDM	23	31	3	45
Control	50	32	2	20
Hispanic				
GDM	43	30	3	41
Control	49	28	2	33

DM, Diabetes mellitus; *GDM*, gestational onset diabetes mellitus.

(kg)/height (m)^2. A body mass index ≥24 was considered overweight. Because patients with gestational onset diabetes mellitus and control subjects were matched for race, racial groups were pooled in the latter analysis.

Results

Ninety-three patients with gestational onset diabetes mellitus and 146 control subjects were enrolled for study between May 1985 and December 1986. The numbers of subjects, mean ages at delivery and numbers of pregnancies, and proportion of subjects with a first-degree relative with diabetes mellitus are shown in Table I for each ethnic group. Mean age and number of pregnancies were similar among the three ethnic groups. Our observation of increased frequency of diabetes mellitus among first-degree relatives of patients with gestational onset diabetes mellitus compared with control subjects is consistent with other reports.[3, 21]

Genotype frequencies did not differ among subjects, within each ethnic group, or in blood analysis stratifying by ethnic group ($\chi^2_{mh} = 0.007$; $p > 0.95$; Table II). However, gene frequencies differed among phenotypic subclasses of gestational onset diabetes mellitus (Table III). The frequency of the class 1 allele was increased among patients with gestational onset diabetes mellitus in group 3 (elevated fasting plasma glucose, nonoverweight) compared with their matched control subjects ($Z = 1.55$, $p = 0.061$). The relative risk associated with the 1,1 genotype in group 3 gestational onset diabetes mellitus was 3.38, although this value did not reach statistical significance in this small sample ($\chi^2_{mh} = 2.26$, $p > 0.10$). The gene and genotype frequencies did not differ significantly among patients with gestational onset diabetes mellitus in groups 1, 2, or 4 compared with matched control subjects.

Of interest is that the increased prevalence of first-degree relatives with diabetes mellitus of patients with gestational onset diabetes mellitus is only evident among those patients with elevated fasting plasma glucose levels. Among patients with gestational onset di-

Table II. Genotype and allele frequencies for insulin gene polymorphism in patients with gestational onset diabetes mellitus and control subjects

	*Genotype**						*Allele class‡*		
	1,1†	*1,2*	*1,3*	*2,2*	*2,3*	*3,3*	*1*	*2*	*3*
Black									
GDM	0.44	0.19	0.33	—	0.04	—	0.70	0.11	0.19
Control	0.46	0.21	0.23	0.04	0.02	0.04	0.68	0.16	0.17
White									
GDM	0.52	—	0.48	—	—	—	0.76	—	0.24
Control	0.54	—	0.38	—	—	0.08	0.73	—	0.27
Hispanic									
GDM	0.51	0.02	0.40	—	0.02	0.04	0.72	0.02	0.26
Control	0.52	0.02	0.42	—	0.02	0.02	0.74	0.02	0.24

*Genotype frequencies calculated as: No. of individuals with genotype/no. of individuals in group.
†1,1 Genotype frequency, patients with gestational onset diabetes mellitus versus control: $\chi^2_{mh} = 0.0007$, $p < 0.50$.
‡Allele frequencies calculated as: No. of each class allele/total no. of alleles.

Table III. Allele and genotype frequencies in the insulin gene hypervariable region and sample characteristics in phenotypic subclasses of gestational onset diabetes mellitus

	Phenotype			*Frequency*				
Group	*FPG*	*BMI*	*N*	*Class 1 allele*	*1,1 genotype*	*Mean age (yr)*	*Mean no. of pregnancies*	*First-degree relative with DM (%)*
1	<105	<24	19	0.68	0.42	31.4	3.2	26
2	<105	≥24	22	0.75	0.57	31.6	3.1	18
3	≥105	<24	12	0.88*	0.75	32.5	3.7	55
4	≥105	≥24	33	0.67	0.42	29.9	3.1	66

FPG, Fasting plasma glucose; *BMI*, body mass index; *DM*, diabetes mellitus.
*$Z = 1.55$, $p = 0.061$.

abetes mellitus with normal fasting plasma glucose levels ($n = 41$), the proportion of first-degree relatives with diabetes mellitus was not different from that of matched controls ($p > 0.80$), and was significantly lower than in patients with gestational onset diabetes mellitus with elevated fasting plasma glucose levels ($n = 46$, $p < 0.001$).

Comment

Our data continue to support observations of others that gestational onset diabetes mellitus is a heterogeneous disorder with respect to both phenotypic and genotypic characteristics.[2-4] Our observation of increased frequencies of the class 1 allele in nonoverweight patients with gestational onset diabetes mellitus with elevated fasting plasma glucose values suggests that this allele may contribute to the development of gestational onset diabetes mellitus in some women. It is of interest that the frequency of the class 1 allele and the 1,1 genotype in these women is comparable with the frequencies reported in type 1 diabetes mellitus.[13] Although our sample size is too small to draw definitive conclusions, the data suggest that these women may represent a subgroup of gestational onset diabetes mellitus with slowly evolving type 1 diabetes mellitus or with undiagnosed type 2 diabetes mellitus before pregnancy. It is tempting to speculate that these women will remain diabetic under nongravid conditions. Lack of associations with the hypervariable region genotypes in the other phenotypic subgroups of gestational onset diabetes mellitus suggests that women who demonstrate glucose intolerance only during pregnancy may represent a novel subgroup of patients with diabetes mellitus whose genetic profiles differ from type 1 and type 2 diabetes mellitus.

Genetic heterogeneity within gestational onset diabetes mellitus is further supported by the different patterns of family histories of diabetes mellitus among phenotypic subclasses of gestational onset diabetes mellitus. That a patient with gestational onset diabetes mellitus with normal fasting plasma glucose values does not show greater prevalences of diabetes mellitus among first-degree relatives as compared with controls raises the possibility that the etiology of gestational onset diabetes mellitus among these women may not be genetic. Alternatively, a gene conferring susceptibility to glucose intolerance only during pregnancy would have sex-limited expression. This gene could be transmitted

through phenotypically normal fathers. Furthermore, because most women with gestational onset diabetes mellitus are asymptomatic, gestational onset diabetes mellitus may not have been recognized in the mothers of our subjects. Thus the prevalence of affected relatives would not necessarily reflect the prevalence of the gestational onset diabetes mellitus susceptibility gene in family members. In either case, our data suggest that in most women the insulin gene hypervariable region does not confer susceptibility to abnormal glucose tolerance in pregnancy.

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Percutaneous umbilical transfusion in severe rhesus isoimmunization: Resolution of fetal hydrops

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The rhesus-sensitized fetus with the worst prognosis is one with early onset of hydropic changes. Percutaneous umbilical blood sampling now enables access to the fetal circulation and thereby allows more precise evaluation of fetal anemia and direct intravascular transfusion. A variation of this technique was used in three pregnancies complicated by fetal pericardial effusion, scalp edema, and abdominal ascites before 26 weeks' gestation. Twelve ultrasound-guided percutaneous transfusions of 30 to 85 ml packed red blood cells were administered into the umbilical cord at its placental insertion. In each fetus the hydropic changes completely resolved and pregnancy outcome was successful. Neither adjunctive therapy with digoxin or Lasix nor exchange transfusions were used. Percutaneous umbilical transfusions appear to have the potential to improve the prognosis for the severely isoimmunized fetus. (Am J Obstet Gynecol 1987;157:1369-75.)

Key words: Percutaneous umbilical transfusion, rhesus isoimmunization, fetal hydrops

The improved resolution of dynamic ultrasound imaging has enabled more precise antepartum fetal evaluation and treatment. For example, recent reports have described the technique of percutaneous umbilical blood sampling and its application for in utero diagnosis and therapy.[1-7] For the severely isoimmunized fetus, the ability to perform a direct intravascular transfusion would eliminate the erratic and incomplete absorption of red blood cells inherent with the traditional intraperitoneal approach. This percutaneous technique could also reduce the procedure-related fetal mortality observed with intraperitoneal transfusions[8] and would seem to be technically easier and less traumatic than either intrahepatic[3] or fetoscopically directed[9] intravascular transfusions. We describe three rhesus-sensitized pregnancies complicated by fetal hydrops before 26 weeks' gestation in which ultrasound-guided percutaneous umbilical transfusions led to the resolution of hydropic changes and successful outcomes. Percutaneous umbilical transfusions appear to have the capability to improve considerably the prognosis for the severely isoimmunized fetus.

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Transfusion technique

Before each procedure, intramuscular meperidine and diazepam plus local anesthesia were administered, but neither prophylactic antibiotics nor tocolytic drugs were used. The placental insertion of the umbilical cord was identified by both linear array and sector real-time ultrasound (General Electric RT 3600, Milwaukee, Wisconsin). A 22-gauge 5-inch (12.70 cm) disposable spinal needle (Becton Dickinson, Rutherford, New Jersey) was then directed to the umbilical cord insertion site (Fig. 1) by the use of a 5 MHz sector transducer with a detachable needle guide (Civco Industries, Kalona, Iowa). After needle tip placement (preferably into the umbilical vein), blood was aspirated for immediate hematocrit analysis as well as complete blood count, blood type and Rh factor, and Kleihauer-Betke test as deemed necessary. The transfusion volume needed to raise the hematocrit to 35% to 45% was calculated by the following formula[9]:

$$V_2 = \left(\frac{Hct_3 - Hct_1}{Hct_2}\right) \times EFW \times V_1$$

where V_1 is the fetoplacental blood volume (150 ml/kg),[10] V_2 is the transfusion volume (in milliliters), EFW is the estimated fetal weight (in kilograms), Hct_1 is the initial fetal hematocrit, Hct_2 is the donor hematocrit (approximately 85%), and Hct_3 is the desired fetal hematocrit. Type O, Rh-negative, cytomegalovirus antibody–negative, frozen, washed, packed red blood cells were transfused at a rate of 1 to 3 ml/min. Fetal blood was also sampled before the end of the procedure to assess the adequacy of therapy.

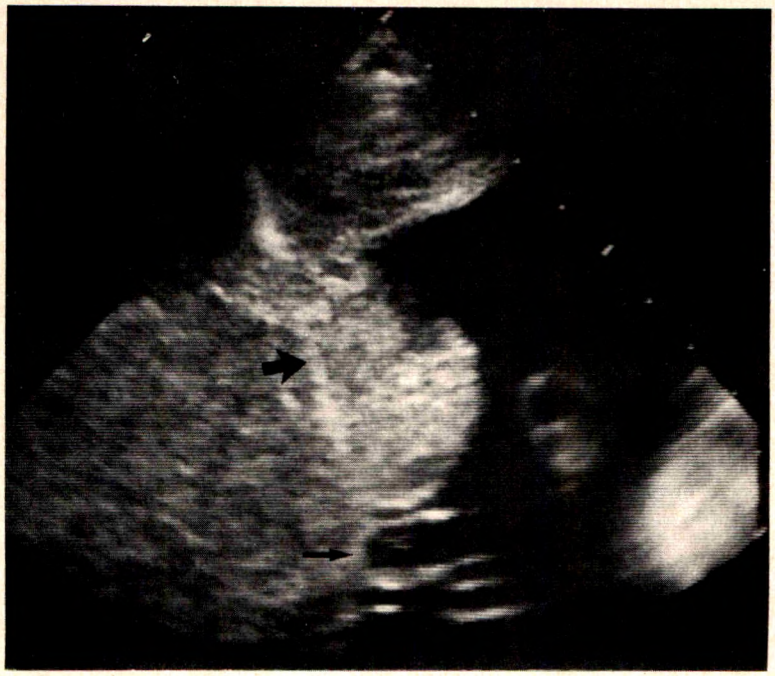


Fig. 1. The needle *(large arrow)* can be seen traversing the anterior placenta and entering the umbilical cord at its placental insertion *(small arrow)*.

Table I. Timing and volume of percutaneous umbilical transfusions in three severely isoimmunized pregnancies

Patient	*Gestational age (wk, day)*	*Transfusion volume (ml)*	*Hematocrit (%)*	
			Pretransfusion	*Posttransfusion*
P. N.	24, 6	30	13	24
	25, 6	58	8	31
	26, 6	55	24	40
V. D.	25, 6	58	14	38
	28, 2	85	10	35
	30, 2	75	21	43
R. G.	22, 6	45	7	30
	24, 1	45	25	50
	26, 6	32	31	45
	28, 6	40	31	44
	30, 6	42	32	NA
	33, 2	40	31	39

NA, Not available.

After the transfusion, uterine contractions and fetal heart rate were monitored for at least 2 hours, after which the patient was discharged. Subsequent fetal surveillance consisted of ultrasound examinations and nonstress tests on at least a weekly basis, plus daily fetal movement counts.

The details of each case are presented below and summarized in Table I.

Case reports

Case 1. P. N., a 28-year-old, gravida 3, para 1-1-0-1, type A, Rh-negative, white woman, had a previous intrauterine fetal death at 34 weeks' gestation. At 19 weeks and 3 days of gestation of the index pregnancy, the indirect Coombs titer was 1:2048 and amniocentesis revealed the amniotic fluid delta optical density at 450 nm (ΔOD_{450}) to be 0.09. Amniocentesis was repeated at 21 weeks and 6 days, at which time the ΔOD_{450} was again 0.09. The patient was then referred to Prentice Women's Hospital, where at 23 weeks and 5 days the ultrasound examination suggested early fetal scalp edema, abdominal ascites, pericardial effusion, and hydramnios. The placenta was anterior and the umbilical cord insertion was clearly visualized in the lower portion of the placenta to the right of midline.

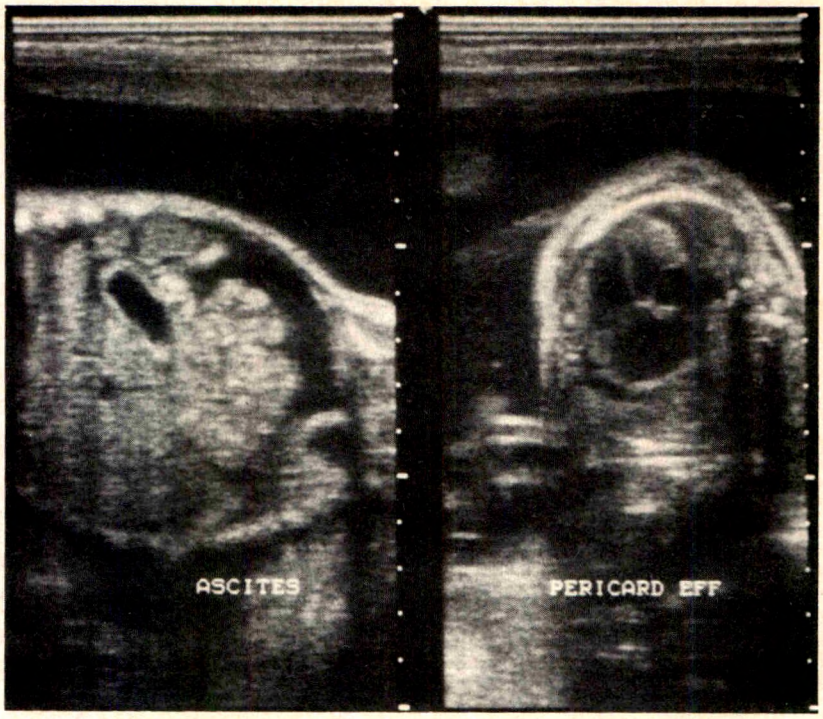


Fig. 2. Abdominal ascites *(left)* and pericardial effusion *(right)* are present at 24 weeks 6 days of gestation.

The initial fetal hematocrit was 24% and the transfusion volume needed to achieve a hematocrit of 35% to 45% was calculated to be 20 ml. After infusion of 18 ml of packed red blood cells, fetal movement dislodged the needle but replacement was not attempted because the calculated volume had nearly been transfused. However, the red blood cell indices and blood type were shortly thereafter reported as consistent with maternal blood (i.e., mean corpuscular volume of 89 fl, type A, Rh negative) and the Kleihauer-Betke test confirmed that the blood sample was maternal. As the patient was reluctant to repeat the procedure, she was monitored with daily fetal movement counts and twice-weekly ultrasound examinations. At 24 weeks and 6 days of gestation, ultrasound showed increased fetal hydrops (Fig. 2) and percutaneous umbilical blood sampling revealed a hematocrit of 13%. An intravascular fetal transfusion of 30 ml of packed red blood cells was performed, raising the hematocrit to 24%. Turbulence within the umbilical cord was visualized throughout the transfusion, assuring satisfactory needle placement. Also, the mean corpuscular volume of the aspirated blood was reported as 151 fl and the blood type was O, Rh positive, further confirming needle placement into an umbilical vessel. Although the final hematocrit was less than the desired end point, concern about possible volume overload led to termination of our initial transfusion. One week later fetal ascites had increased and a sinusoidal fetal heart rate pattern was noted. Intravascular transfusion was repeated, at which time the Kleihauer-Betke test revealed <1% fetal cells; 58 ml of packed red blood cells raised the fetal hematocrit from 8% to 31%. Fetal movement caused dislodgment of the needle twice during the transfusion, as evidenced by turbulence in the amniotic fluid rather than the umbilical vessel, and reinsertion of the needle was required. By the end of the transfusion the sinusoidal pattern had disappeared and fetal heart rate reactivity had improved. The following week, at 26 weeks and 6 days of gestation, ultrasound examination indicated that the hydramnios had resolved and that the fetal hydrops had improved. A third transfusion was performed, during which 55 ml of packed red blood cells raised the hematocrit from 24% to 40%.

Three days later, at 27 weeks and 2 days of gestation, the patient presented to labor and delivery in premature labor. Ultrasound demonstrated complete resolution of fetal hydrops. Tocolysis with intravenous ritodrine was attempted, but spontaneous rupture of the membranes occurred, followed by umbilical cord prolapse. A primary cesarean section was performed, with delivery of a 1080 gm female infant with Apgar scores of 2 and 7 at 1 and 5 minutes, respectively, and an umbilical artery pH of 7.29. The umbilical cord showed no evidence of trauma. A maternal urine toxicology screen was positive for phencyclidine, but the newborn urine screen was negative. The neonatal hematocrit was 37%, the white blood cell count was 6700/mm^3, the platelet count was 252,000/mm^3, and the Kleihauer-Betke test identified <1% fetal cells. The infant developed respiratory distress syndrome and remained intubated for 14 days but has since done well. Although

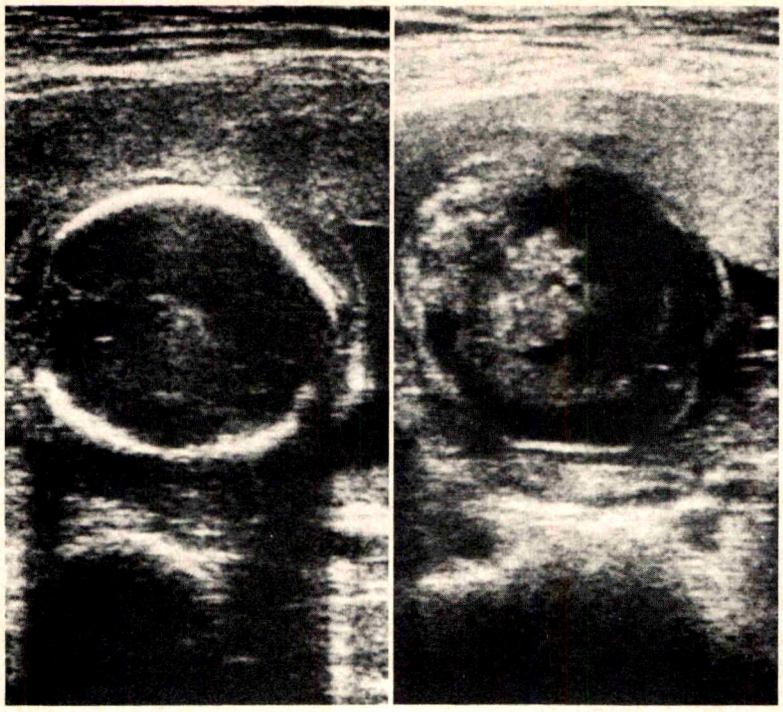


Fig. 3. Fetal scalp edema *(left)* and massive abdominal ascites *(right)* surrounding the liver and bowel are clearly visualized at 22 weeks 5 days of gestation.

exchange transfusions were not required, a total of 156 ml of packed red blood cells was transfused on 10 occasions during the 79-day hospitalization, principally to replace iatrogenic losses. On discharge the hematocrit was 25% and the reticulocyte count remained 0.0%. No outpatient transfusions were administered but the reticulocyte count did not rise to 0.5% until day 101 of life. By day 107 the reticulocyte count was 4.2%, with the peak being 10.1% on day 114.

Case 2. V. D., a 32-year-old, gravida 4, para 0-2-1-0, type O, Rh-negative, black woman, had a history of stillbirths at 36 weeks' and 26 weeks' gestation, the latter after an intraperitoneal fetal transfusion. During the current pregnancy the initial indirect Coombs titer was 1:64 and amniocentesis at 23 weeks 6 days of gestation identified the ΔOD_{450} to be 0.40. Ultrasound examination could not clearly identify the umbilical cord insertion into the anterior fundal placenta, and in view of the absence of fetal hydrops plus the circumstances of the last pregnancy loss, it was elected to delay the transfusion until the following week rather than perform an intraperitoneal procedure. At 24 weeks 6 days, percutaneous umbilical blood sampling was unscucessfully attempted, but with the continued absence of hydropic changes the patient was followed up with daily fetal movement counts and ultrasound evaluation twice weekly. At 25 weeks 6 days, ultrasound examination revealed mild scalp edema, ascites, and pericardial effusion. Percutaneous umbilical blood sampling was successful on the fourth attempt and was accomplished by traversing the placenta and amniotic cavity, thereby entering the umbilical cord 2 to 3 cm from the placental insertion. The fetal hematocrit of 14% was raised to 38% by the transfusion of 58 ml of packed red blood cells. Ultrasound examination during the next week indicated that the hydrops had resolved, but at 28 weeks 2 days of gestation mild hydropic changes recurred and hydramnios was present. The fetal hematocrit was only 10%, with <1% fetal cells, and was raised to 35% via the transfusion of 85 ml of packed red blood cells. At 29 weeks of gestation ultrasound demonstrated resolution of the fetal hydrops and hydramnios, but at 30 weeks 2 days the patient noted decreased fetal movement and the nonstress test, which had previously been reactive, was now considered borderline. A third intrauterine transfusion was therefore performed, at which time the pretransfusion hematocrit of 21% was raised to 43% by the infusion of 75 ml of packed red blood cells. Amniotic fluid was aspirated during the procedure and the lecithin/sphingomyelin ratio was later reported as 2.0 with phosphatidylglycerol present.

In view of the previous declines in fetal hematocrit during a 2-week interval and the mature lung profile, it was decided to deliver the fetus at 32 weeks 3 days of gestation. A 1700 gm male infant was delivered by primary cesarean section with Apgar scores of 7 and 8 at 1 and 5 minutes, respectively, and an umbilical artery pH of 7.32. The umbilical cord was centrally inserted into the placenta and demonstrated no evidence of trauma. The neonatal hematocrit was 39%, the white blood cell count was 5600/mm^3, the platelet count was 284,000/mm^3, and the Kleihauer-Betke test revealed

<1% fetal cells. The neonate was extubated within 24 hours, required no exchange transfusions, and has done well. Only two simple transfusions of 16 and 22 ml of packed red blood cells were administered during the 26-day hospitalization. At discharge the neonatal hematocrit was 42%, but by 8 weeks of age the hematocrit had dropped to 20% with the reticulocyte count still at 0.0%. An outpatient transfusion raised the hematocrit to 35%. Two weeks later the reticulocyte count was 1.8% and no further transfusions were required.

Case 3. R. G., a 38-year-old, gravida 3, para 2-0-0-2, type O, Rh-negative, white woman, was noted to be sensitized at the time of her first delivery, which was by cesarean section because of cephalopelvic disproportion. Her second delivery was by repeat cesarean section at 38 weeks' gestation; the umbilical cord hemoglobin and bilirubin levels were 11.5 gm/dl and 6.7 ng/dl, respectively, and the neonate required seven exchange transfusions. She was first seen at Prentice Women's Hospital at 9 weeks' gestation of the index pregnancy, at which time the indirect Coombs titer was 1:512. Thought was given to performing percutaneous umbilical blood sampling at 18 weeks for both rapid determination of karyotype and assessment of fetal hematocrit. However, it was felt that the risks of this relatively new procedure would be greater than those of genetic amniocentesis, particularly in a patient who had two previous term pregnancies and might not require intrauterine transfusion. Therefore genetic amniocentesis was performed at 15 weeks and revealed a normal karyotype and α-fetoprotein level. Unfortunately, at 22 weeks 5 days of gestation, ultrasonic examination identified fetal scalp edema and massive abdominal ascites (Fig. 3) without hydramnios. On the next day an ultrasound-guided intrauterine transfusion was performed, with the umbilical vein penetrated at its insertion into the anterior placenta. Forty-five milliliters of packed red blood cells raised the fetal hematocrit from 7% to 30%. One week later, at 23 weeks 6 days of gestation, minimal scalp edema and pericardial effusion were present but moderate abdominal ascites persisted. An attempt to repeat the intrauterine transfusion was unsuccessful, principally because the fetus was lying against the placenta at the site of the umbilical cord insertion. However, 2 days later, at 24 weeks 1 day, a repeat transfusion was performed without difficulty, during which 45 ml of packed red blood cells raised the fetal hematocrit from 25% to 50%; a Kleihauer-Betke test performed on the initial sample indicated <1% fetal cells. By 25 weeks' gestation all signs of fetal hydrops had resolved and a third transfusion of 32 ml of packed red blood cells at 26 weeks 6 days raised the fetal hematocrit from 31% to 45%. At 28 weeks 6 days the fetus was again lying against the umbilical cord insertion, but the fourth transfusion was easily performed by rotating the patient approximately 45 degrees onto her left side. Forty milliliters of packed red blood cells were infused and raised the fetal hematocrit from 31% to 44%. The fifth transfusion was performed at 30 weeks 6 days of gestation, with the initial fetal hematocrit being 32%. Forty-two milliliters of packed red blood cells were transfused, but on an attempt to obtain a posttransfusion blood sample the needle was displaced and could not be reinserted into the umbilical vein. Amniotic fluid was aspirated and yielded a lecithin/sphingomyelin ratio of 1.6 with phosphatidylglycerol absent. After the transfusion the baseline fetal heart rate declined from 140 to 110 bpm but the heart rate pattern remained reactive and the patient was allowed to go home. Two days later a nonstress test was reactive, with a baseline heart rate of 140 bpm. At 32 weeks 5 days, amniocentesis was performed, but the lecithin/sphingomyelin ratio was reported as 1.4 with phosphatidylglycerol absent. Consequently, a sixth transfusion was administered at 33 weeks 2 days of gestation and 40 ml of packed red blood cells raised the fetal hematocrit from 31% to 39%. Again there was a decrease in baseline fetal heart rate from 130 to 105 bpm, but by 2 hours the fetal heart rate returned to 130 bpm and the tracing was reactive.

At 35 weeks 5 days, the lecithin/sphingomyelin ratio was 3.0 with phosphatidylglycerol present. The next day a 2260 gm female infant was delivered by repeat cesarean section with Apgar scores of 7 and 9 at 1 and 5 minutes, respectively, and an umbilical artery pH of 7.30. The neonatal hematocrit was 31%, the white blood cell count was 12,900, the platelet count was 213,000, and the Kleihauer-Betke test indicated <1% fetal cells. The newborn infant was discharged with her mother on the fifth hospital day; the hematocrit was 33% and the reticulocyte count reported at <0.2%. On day 15 of life an outpatient transfusion of 50 ml of packed red blood cells was administered because the hematocrit was 21% and the reticulocyte count was <0.1%. On day 36, 30 ml of packed red blood cells were transfused because the hematocrit was 28%. By day 49 the hematocrit dropped to 23%, with the reticulocyte count persisting at <0.1%, but six days later the reticulocyte count increased to 5.5%, with a peak of 11.2% on day 59. No further transfusions were necessary.

Comment

Therapy for the fetus with severe rhesus isoimmunization is directed toward correction of the hemolytic anemia. In addition to eliminating the problem of incomplete absorption of blood and potentially decreasing the risks of fetal morbidity and mortality associated with intraperitoneal therapy, the percutaneous umbilical technique enables the fetal hematocrit to be determined during the transfusion. This maximizes the ability to raise the fetal hematocrit to the desired value and minimizes the possibility of either undertransfusion or overtransfusion. Even more basic to the management of the isoimmunized pregnancy, percutaneous umbil-

ical blood sampling can be used in conjunction with the Liley curves, particularly in the second trimester, to determine whether the optimal management is continued observation or intrauterine transfusion.[11]

Adjunctive therapy with furosemide, digoxin, or both has been recommended at the time of fetal transfusion.[9, 12] However, it is doubtful that the erythroblastosis is secondary to heart failure, because even the severely hydropic fetus does not appear to have increased intravascular volume.[13] More likely, the hydropic changes are related to the increase in portal pressure because of disordered hepatic parenchyma and the decrease in colloid osmotic pressure associated with hypoproteinemia secondary to liver dysfunction.[8] Chronic anemia may also be etiologically important, and it has recently been postulated that this may initiate the hydropic process via tissue hypoxia.[14] Consequently, furosemide may unnecessarily deplete the intravascular volume and digoxin will probably not be beneficial, because even if heart failure is present it likely will be high-output failure related to anemia.[14, 15] Correlation of the anemia and suppression of extramedullary hematopoiesis treat the underlying disease process, and in the three patients we report these procedures successfully reversed the hydropic changes. These observations would seemingly suggest that adjunctive therapy during intravascular transfusion is unnecessary.

The theoretical risk of cardiovascular compromise from fluid overload with simple transfusions as opposed to exchange transfusions has been raised.[6] However, in these three pregnancies the hydropic fetuses tolerated the simple transfusions of packed red blood cells in the volumes necessary to increase the fetal hematocrit above 35%. During the 12 transfusions, neither fetal tachycardia nor periodic decelerations were observed, and the fetal heart rate reactivity was either unchanged or improved after transfusion. The two instances in which the baseline heart rate declined from 130 to 140 down to 105 to 110 bpm probably reflected baroreceptor reflex activity, as the heart rate pattern remained reactive and the change was well tolerated by the fetus. Of note, this downward resetting of the fetal baseline heart rate occurred in the same fetus at the most advanced gestational ages at which it was transfused. A possible disadvantage of exchange transfusions is that the likelihood of needle displacement will be increased with prolongation of the procedure.

Successful transfusions with a free-hand technique with either sector or linear-array ultrasound have been previously described.[1, 6, 7] An advantage of the sector transducer with a detachable needle guide is that the needle is fixed in the sonographic plane and the needle tip can be continuously visualized during the puncture of the umbilical vessel and the subsequent transfusion. Placement of the needle in the umbilical vein is preferred because of its larger diameter and decreased propensity for vasospasm, but in one of the 12 transfusions the needle tip was in an umbilical artery. Most importantly, the observation of turbulence within the umbilical vessel during the transfusion ensures proper placement throughout the procedure. The 22-gauge disposable spinal needle is potentially more traumatic than the 25-gauge needle used in one series.[2] However, evidence of umbilical cord trauma was not observed at delivery and Daffos et al.,[1] using a 20-gauge needle, identified only one possible case of cord trauma and fetal death during fetal blood sampling in a series of 606 pregnancies. Disadvantages of the 25-gauge needle are that it is more flexible and more easily bent with maternal or physician movement. Further, the smaller gauge is more likely to cause lysis of cells at the time of transfusion or to become occluded. Finally, the blood was infused at a rate of 1 to 3 ml/min and a smaller bore needle would increase the time required for the procedure.

In summary, although the risks associated with percutaneous umbilical blood sampling and intravascular therapy remain to be quantified and issues pertaining to technique and timing of transfusion need to be resolved, the ability to gain access to the fetal circulation offers considerable promise in both antepartum diagnosis and treatment. In these three severely isoimmunized pregnancies, early onset fetal hydrops was reversed and successful outcomes were achieved. As previously cautioned,[16] this procedure requires considerable skill and precision and should be performed at centers with experience in fetal diagnosis and therapy.

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Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term

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Eighty-three healthy pregnant women between 26 and 40 weeks' gestational age were studied to examine effects of a 5-second external vibratory acoustic stimulus on the fetal heart rate, fetal breathing, and gross fetal body movement patterns. There was an immediate fetal heart rate response, following stimulus, characterized by an increase in duration of fetal heart rate accelerations from 26 weeks to term with an increase in basal fetal heart rate after 30 weeks. There was also a delayed response after 33 weeks' gestation, which consisted of an increase in the number of fetal heart rate accelerations and the incidence of gross fetal body movements for up to 1 hour after a 5-second stimulus with the electronic artificial larynx. Term (36 to 40 weeks) fetuses made breathing movements more irregularly after vibratory acoustic stimulation. We suggest that human fetal responses to stimulation with the electronic artificial larynx may reflect a functional maturation of the fetal central nervous system. (AM J OBSTET GYNECOL 1987;157: 1375-81.)

Key words: Fetal responses to sound and vibration; antepartum fetal heart rate testing; effect of vibration on fetal movements

It has been demonstrated that external vibratory acoustic stimulation with an electronic artificial larynx reproducibly causes changes in the fetal heart rate (FHR)[1-3] and movement patterns[4] in term fetuses. It was proposed that FHR accelerations after stimulation with the EAL might reflect fetal health. Before introduction of this device as a clinical test, there is a need to document the effect of gestational age on the response.

The purpose of this study was to define, in a group of healthy fetuses from 26 weeks' gestational age to term, changes in fetal heart rate, fetal breathing, and gross body movements after a standardized external vibratory acoustic stimulus.

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Patients and methods

Patients. Informed consent was obtained from 89 healthy pregnant women between 26 and 40 weeks' gestational age. Eighty-three women remained in good

health. Five women were delivered of their infants before 37 weeks: Two had abruptio placentae, two had premature labor, and one was delivered of a stillborn infant at 35 weeks'. The cause of death was unknown. Another patient was lost to follow-up. These six women were excluded from subsequent analysis. All other women had normal term deliveries that confirmed gestational age and the good health of the fetuses studied. The average gestational age at delivery was 39.3 ± 0.1 week (SEM). The average weight of the fetuses was 3350 ± 42 gm. The average 5-minute Apgar score was 9.3 ± 0.1. No Apgar score was <7 at 5 minutes. Umbilical cord blood arterial samples were obtained at birth in 58 of the 83 fetuses. The mean umbilical arterial P_{O_2} was 17.8 ± 1.0 mm Hg, the P_{CO_2} was 50.0 ± 1.3 mm Hg, and the pH was 7.27 ± 0.01. Ten (13.7%) women were delivered by cesarean section: Two developed acute fetal distress in labor (5-minute Apgar scores of 8 and 10, respectively), one had cephalopelvic disproportion, one had a transverse fetal lie at the onset of labor, and six had repeat cesarean sections. Eighty-one fetuses were in the vertex presentation and two were in breech positions at the time of delivery.

Experimental design. The women received a standardized 800 kcal breakfast or lunch at 8:00 AM (n = 48) or 12:00 noon (n = 53). All studies began 1 hour later and were conducted in a quiet room with the patients either sitting upright in bed or resting in a lateral recumbent position. Women were asked not to smoke for at least 4 hours before the studies. Smoking or food intake by patients or observers was not permitted during the studies.

All women had been acclimatized by listening to the stimulus in air before the beginning of studies and were informed that the stimulus might be applied either on the abdomen or the hand. Women were studied continuously for 2 hours. At the end of the first hour of observation, a 5-second vibratory acoustic stimulus was applied on the surface of the maternal abdomen over the fetal head (n = 51) or on the maternal hand (n = 50) for controls. The choice of whether to stimulate the abdomen or the hand of each woman was made from random number tables. Eight women in different gestational age groups had both control and stimulus, and studies in these women were separated by at least 2 days. All women received only one stimulus to either the abdomen or the hand during individual studies.

The external vibratory acoustic stimulus used in this study was generated with a Model 5C electronic artificial larynx (Western Electric, New York, New York, courtesy of Mr. John Kasper, Corometrics Medical Systems), as previously described.[1]

Fetal and maternal cardiovascular measurements. The FHR was recorded with a Hewlett Packard Model 8040A external monitor (Hewlett Packard GMBH, Boeblinger, Federal Republic of Germany) and a compact transducer and analyzed on line by a Stage II microprocessor (Sage Technology, Reno, Nevada) as previously described.[3] The fetal heartbeat intervals were examined with an artifact detection algorithm,[5] and valid pulse intervals were averaged over 3.75 seconds. During analysis, a baseline was fitted to the heart rate record[6] and accelerations of ≥10 bpm for ≥15 seconds above the baseline were recognized automatically.

For each 10-minute interval, the basal FHR (in beats per minute), the root mean square (in milliseconds), and the mean minute range (in milliseconds) were calculated. The root mean square and the mean minute range are numerical measurements of long-term FHR variability.[7] The maternal heart rate and the systolic, diastolic, and mean arterial blood pressures were automatically recorded every 5 minutes with a Dinamap (Model 845 XT, Critikon Inc., 1982, Tampa, Florida) and were not altered by vibratory acoustic stimulation.

Measurements of fetal activity. Fetal breathing movements and gross fetal body movements were measured with a real-time ADR ultrasound scanner (Model 2130, Advanced Diagnostic Research Corp., Tempe, Arizona) and analyzed on line in 5-minute intervals with a PDP 11/34 minicomputer as previously described.[8] The transducer operated at a frequency of 3.5 MHz with an average intensity of 0.045 mW/cm^2. Individual gross fetal body movements and fetal breathing movements were identified on a video monitor and coded with an event marker. The transducer was positioned over the maternal abdomen to permit continuous observation of a longitudinal cross section of fetal chest and abdominal wall echoes.[8]

Data analysis. The basal FHR, the number of FHR accelerations of ≥10 bpm for ≥15 seconds above the baseline, the duration and amplitude of individual FHR accelerations, the total time spent in acceleration, the root mean square, and the mean minute range values were determined for successive 10-minute intervals. Maternal heart rate and blood pressure were analyzed as means ± SEM for each 5-minute interval. All other results are presented as means ± SEM for successive 10-minute intervals before and after vibratory acoustic stimulus on the maternal abdomen or on the maternal hand (control) for the 2-hour observation periods. Portions or records that could not be analyzed because of FHR signal loss of more than 30% or patient interruption were not included. These portions made up 3.5% of total study time.

Measurements of the percentage of time spent mak-

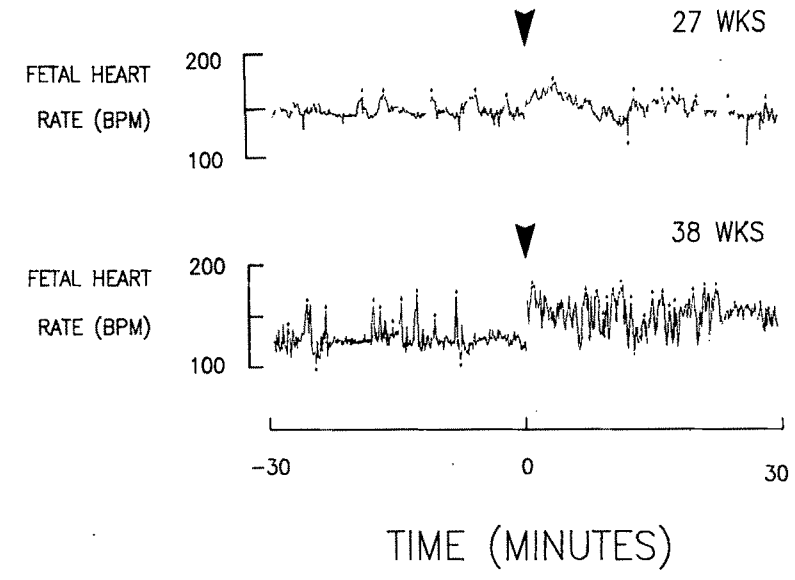


Fig. 1. Examples of an increase in FHR after a 5-second vibratory acoustic stimulus with the electronic artificial larynx in a 27-week fetus and in a 38-week fetus.

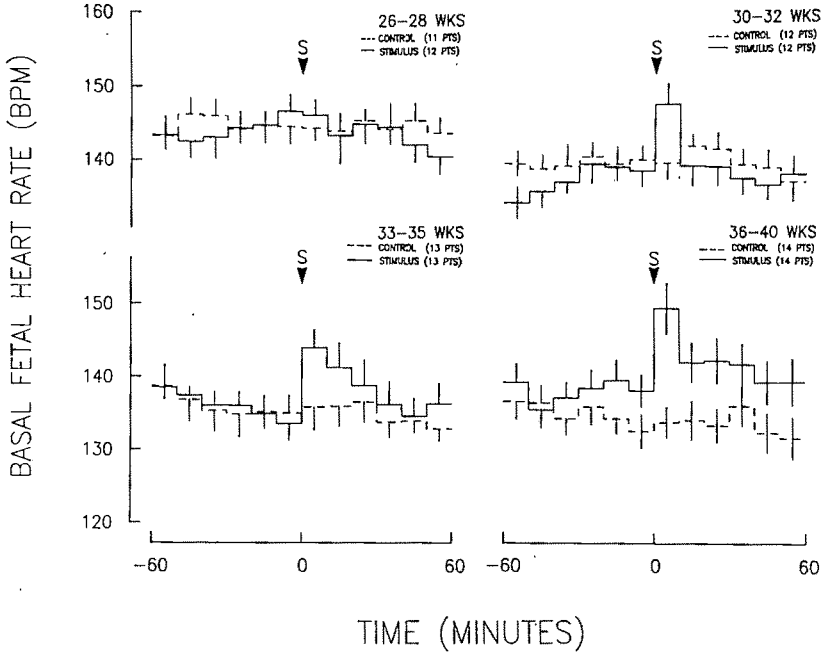


Fig. 2. The mean basal FHR (±SEM) was plotted for each group of fetuses at 26 to 28, 30 to 32, 33 to 35, and 36 to 40 weeks' gestation, in 10-minute intervals before and after control (– – –) or stimulus (———).

ing gross fetal body movements, the percentage of time spent making breathing movements, and the frequency distribution of breath-to-breath intervals were calculated for each 10-minute interval of the 202 hours of total observation time. Portions of movement records that could not be analyzed because of technical problems or patient interruption made up 2.7% of total time.

Statistical significance between groups before and after stimulus or control was determined by using a

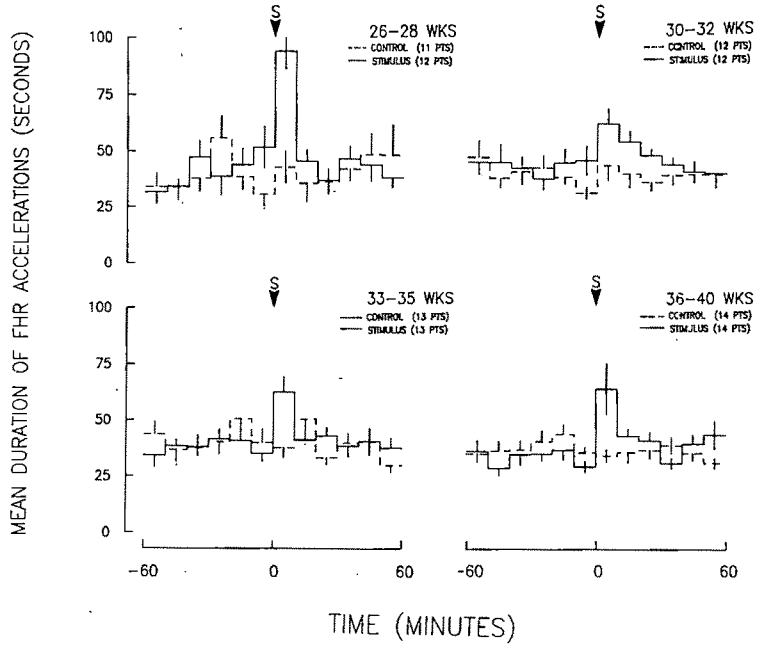


Fig. 3. The mean duration of FHR accelerations was plotted for each group of fetuses at 26 to 28, 30 to 32, 33 to 35, and 36 to 40 weeks' gestation, in 10-minute intervals before and after control (– – –) or stimulus (———).

mixed, nested analysis of variance comparing overall means and each of the 10-minute intervals[9] with an HP 9845B microprocessor (Hewlett Packard, Computer Division, Fort Collins, Colorado).

Results

FHR and FHR accelerations. Examples of the FHR response after vibratory acoustic stimulation with the electronic artificial larynx at 27 and 38 weeks are shown in Fig. 1. The mean basal FHR was 140.0 ± 1.9, 136.0 ± 1.4, and 138.4 ± 2.2 bpm at 30 to 32, 33 to 35, and 36 to 40 weeks of gestation, respectively, during the hour before stimulus. The mean basal FHR increased significantly to 148.0 ± 2.8, 144.0 ± 2.5, and 149.0 ± 2.3 bpm during the first 10 minutes after stimulus compared with control values (analysis of variance; $p < 0.001$, $p < 0.01$, $p < 0.001$, respectively) (Fig. 2). Basal FHR also remained above the control value for the hour after the stimulus in term (36 to 40 weeks) fetuses (Fig. 2). In the 26 to 28 weeks group of fetuses, the basal FHR was 144.4 ± 1.3 bpm before vibratory acoustic stimulation and did not change after either control or stimulus (Fig. 2).

Fig. 3 summarizes the mean duration of FHR accelerations per 10-minute interval from 26 to 40 weeks before and after stimulus or control. Analysis of variance demonstrated a significant increase in the mean duration of FHR accelerations at 26 to 28, 30 to 32, 33 to 35, and 36 to 40 weeks during the first 10 minutes after the stimulus (94 ± 5, 62 ± 6, 63 ± 7, and 64 ± 11 seconds, respectively) compared with control values (40 ± 6, 40 ± 6, 38 ± 3, and 35 ± 1 seconds) (analysis of variance; $p < 0.001$, $p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively).

No significant change was found in computer-derived indexes of FHR variability at any gestational age after vibratory acoustic stimulation when compared with control values.

Relationship between gross fetal body movements and the number of FHR accelerations. There were 2.9 ± 0.2 and 2.5 ± 0.3 FHR accelerations per 10-minute interval at 33 to 35 and 36 to 40 weeks' gestational age during the hour before vibratory acoustic stimulation (Fig. 4, *A* and *C*). Analysis of variance demonstrated a significant increase in the number of FHR accelerations between 10 and 30 minutes at 33 to 35 weeks and between 10 and 20 minutes at 36 to 40 weeks ($p < 0.02$ and $p < 0.03$). It was important that all fetuses that received stimuli from 33 to 40 weeks exhibited FHR accelerations between 10 and 20 minutes after stimulation with the electronic artificial larynx (Fig. 4, *A* and *C*).

Similarly, there was a significant increase in the incidence of gross fetal body movements from 7.4% ± 0.9% and 5.8% ± 0.7% (33 to 35 and 36 to 40 weeks) during the hour before stimulus to 14.5% ± 2.8% and 13.8% ± 2.3% in the hour after stimulus with electronic artificial larynx (analysis of variance; $p < 0.01$ and $p < 0.001$, respectively) (Fig. 4, *B* and *D*). The maximum increase in the incidence of gross fetal body

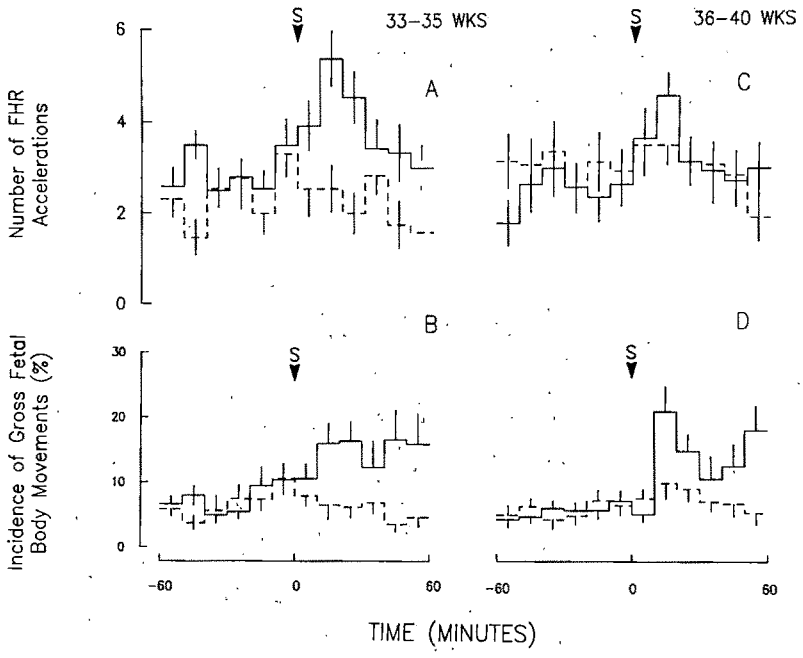


Fig. 4. The mean number of FHR accelerations was plotted in 10-minute intervals from 33 to 40 weeks before and after control (– – –) or stimulus (———). The mean incidence of gross fetal body movements was also plotted on the same time scale. There was a delayed increase in both number of FHR accelerations and the incidence of gross fetal body movements after vibratory acoustic stimulation.

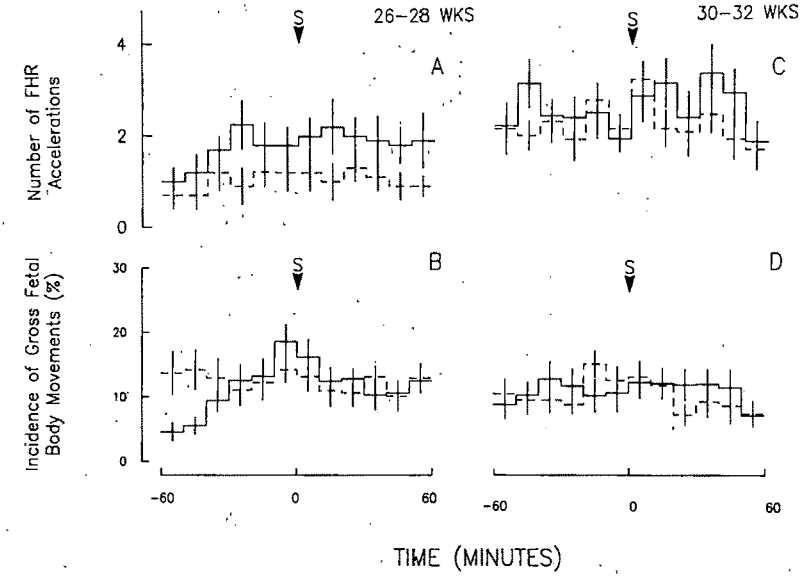


Fig. 5. The mean number of FHR accelerations was plotted in 10-minute intervals from 26 to 32 weeks before and after control (– – –) or stimulus (———). The mean incidence of gross fetal body movements was also plotted on the same time scale.

movements occurred between 10 and 30 minutes after the 5-second stimulus in both groups and did not return to control values for 30 to 60 minutes. The peak in the number of FHR accelerations coincided with the peak in the incidence of gross fetal body movements. Neither the number of FHR accelerations nor the incidence of gross fetal body movements increased until 10 minutes after the 5-second stimulus (Fig. 4, *A* to *D*). The number of FHR accelerations and the incidence of gross fetal body movements did not change significantly after

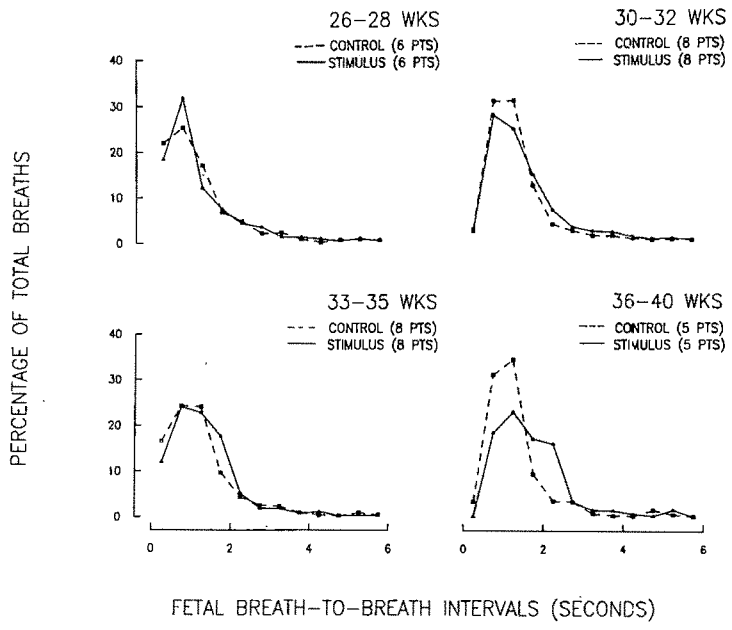


Fig. 6. Fetal breath-to-breath intervals were measured, and the histograms of the mean percentage of total breath-to-breath intervals in 0.5-second increments were plotted for 54 fetuses after stimulus (———) or control (– – –). There was a broad distribution of breath-to-breath intervals at 1.0 to 2.5 seconds without a well-defined peak after 36 weeks' gestation.

stimulus or control at 26 to 28 and 30 to 32 weeks' gestation (Fig. 5, *A* to *D*).

Fetal breathing movements. Term (36 to 40 weeks) fetuses made breathing movements 37.4% ± 5.2% of the time before the stimulus and decreased significantly to 15.7% ± 5.4% during the hour after stimulation with the electronic artificial larynx as compared with control values (31.4% ± 5.1%) (analysis of variance, $p < 0.05$). The incidence of fetal breathing movements was 17.1% ± 4.2%, 34.2% ± 5.1%, and 34.5% ± 5.2% during the hour before stimulus at 26 to 28, 30 to 32, and 33 to 35 weeks, respectively, and was not significantly altered by stimulus when compared with control.

Fig. 6 demonstrated histogram plots of the mean percentage of total breath-to-breath intervals in successive 0.5-second increments in 54 fetuses during the hour after control (n = 27) or stimulus (n = 27) between 26 and 40 weeks. At 36 to 40 weeks, there was a skewed distribution of breath-to-breath intervals with a peak of 1.0 to 1.5 seconds during the hour after control, but during the hour after stimulus, there was a broad distribution of breath-to-breath intervals at 1.0 to 2.5 seconds without a well-defined peak (Fig. 6). This suggested that term (36 to 40 weeks) fetuses were breathing more irregularly after vibratory acoustic stimulation. No such change in breath-to-breath intervals was found before 36 weeks' gestational age (Fig. 6).

Comment

The data demonstrated that, in a group of highly selected healthy fetuses from 26 to 40 weeks, human fetal responses to vibratory acoustic stimulation were significantly altered by gestational age. There was an immediate FHR response characterized by an increase in duration of FHR accelerations from 26 weeks to term with an increase in basal FHR after 30 weeks. There was also a delayed response after 33 weeks' gestation, which consisted of (1) an increase in the number of FHR accelerations and (2) an increase in the incidence of gross fetal body movements for up to 1 hour after application of a 5-second stimulation with the electronic artificial larynx. In term fetuses, there was an immediate decrease in the incidence of fetal breathing movements with a more irregular breathing pattern that persisted for 1 hour after stimulus.

An increase in FHR after external vibratory acoustic stimulation with a loudspeaker generating a white noise at 110 dB in an anencephalic fetus has been previously reported.[10] This suggested that the origin of the immediate FHR response after vibratory acoustic stimulation is subcortical rather than coming from the cerebral cortex. In this study, four healthy fetuses did not respond to this stimulus: two at 26 weeks', one at 27 weeks', and one at 30 weeks' gestation. However, a consistent FHR response was obtained after 30 weeks. This supported the concept that the immediate increase

in basal FHR and duration of FHR accelerations after stimulation with the electronic artificial larynx might be related to functional maturation of the fetal autonomic nervous system, which is believed to occur during a critical and narrow period between 28 to 30 weeks' gestational age.[11]

The delayed increase in the number of FHR accelerations with an increase in the incidence of gross fetal body movements after the 5-second stimulus, which occurred after 33 weeks' gestation, was very similar to the wakefulness state described by Prechtl et al.[12] in newborn humans at 34 to 36 weeks and by Nijhuis et al.[13] in human fetuses after 36 weeks. The irregular breathing pattern seen after stimulus at >36 weeks' gestation could also reflect a state of wakefulness.[12]

Smith et al.[14] recently reported that 47% of fetuses did not have any FHR accelerations after stimulation with the electronic artificial larynx during labor with normal scalp pH values compared with 9% during antepartum FHR testing.[2] These results supported the hypothesis that the immediate FHR response after vibratory acoustic stimulation might be markedly attenuated during labor and after rupture of the membranes because of impairment of transmission of the vibrational component produced by the electronic artificial larynx.[1]

Meissner's corpuscles, which transmit cutaneous vibratory tactile sensation, with a fundamental frequency response of 80 Hz, have been anatomically identified between 24 and 28 weeks in human fetuses.[15] Leader et al.,[16] using a vibratory tactile stimulus with a fundamental frequency of 98 Hz, were able to elicit a fetal "startle reflex" as early as 24 weeks' gestation. The spectrum analysis of sound and vibration produced by the electronic artificial larynx demonstrated a sound pressure level of 95 dB at 100 Hz and an amplitude of vibration of 43 dB at 100 Hz.[1] It is therefore possible that sound and vibration produced by the electronic artificial larynx at low frequencies (≤100 Hz) are enhanced with transmission through the uterine wall as reported in sheep[17] and stimulated vibratory tactile Meissner's corpuscles. Whether this mechanism could explain significant changes in rest-activity cycles in human fetuses after stimulation with the electronic artificial larynx remains speculative. However, stimulation of Meissner's corpuscles rather than acoustic stimulation could explain the 47% of false positive stimulation tests with the electronic artificial larynx during labor[14] and the lack of any correlation between congenital deafness and the FHR response to vibratory tactile stimulation.[10]

The results indicated that human fetal responses after vibratory acoustic stimulation were altered by gestational age. We suggest that these changes reflect a functional maturation of the fetal central nervous system and are mediated by vibrational pathways. It will be important to determine if fetal responses after stimulation with the electronic artificial larynx can be altered in compromised sick fetuses or in fetuses with subsequent developmental delay.

We wish to thank Professor G. S. Dawes and Mr. Isaac Rapoport for their interest in this work.

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Fetal auditory brain stem response: Effect of increasing stimulus rate during functional auditory development

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The fetal auditory brain stem response to increasing stimulus rates of 16, 32, 45, 64, 87, and 100 clicks/sec were determined in eight fetal lambs of 115 to 136 days' gestation (term = 145 days). To conduct these studies, a hysterotomy was performed on the pregnant ewe at 110 to 118 days' gestation to implant stainless steel electrodes in the fetal scalp and a hearing aid receiver in the fetal external ear canal. The fetus was returned to the uterus and the pregnancy was allowed to continue. Auditory brain stem responses from younger fetuses (115 to 125 days' gestation) were present at the slower click rates but were not observed consistently at the fast click rates until the fetuses were older (125 to 136 day's gestation). Latency values of the auditory brain stem response peaks decreased with increasing fetal age and increased with increasing stimulus rates. From a two-way analysis of variance, the influence of age and click rate alone and more important, the interaction of age and click rate were determined to have highly significant effects ($p < 0.001$) on latency values of wave I, wave V, and central conduction time. These auditory brain stem response results suggest that the cochlear component of the fetal auditory system undergoes rapid change in late gestation to process incoming stimuli at a faster rate. (AM J OBSTET GYNECOL 1987;157:1382-7.)

Key words: Fetal auditory brain stem response, click rate, auditory development, fetal sheep

Previous reports from our laboratory have demonstrated that the fetal auditory brain stem response can be measured in utero in the chronically instrumented fetal lamb during the final weeks of pregnancy.[1-3] These reports indicate that (1) the fetus responds neuroelectrically to sound, (2) auditory function in the fetal lamb begins at 115 to 120 days' gestation, and (3) developmental changes in auditory function can be monitored during the final weeks of pregnancy. This fetal animal model may have applications in testing the effects of a variety of perinatal conditions such as hypoxia, acidosis, infection, and exposure to drugs on the developing fetal auditory system.

Congenital hearing loss has been associated with a variety of causes including maternal infection,[4] prematurity,[5] acidosis,[5] and anoxia.[6] The perinatal history of an infant or young child with congenital hearing loss is often examined to identify adverse perinatal events as the causative agent for the hearing loss. Newborn hearing loss is linked by association to adverse perinatal events, since no animal model has been available to test adequately perinatal causes of congenital hearing loss. Defining normal auditory development in the chronically instrumented fetal sheep model may indicate critical periods of fetal growth in which the auditory system is vulnerable to perinatal insults.

In the present study, we examined the effects of increasing stimulus rate on the auditory brain stem response of the developing fetal lamb. The purposes of this study were as follows: (1) to determine if the immature auditory system of the fetus responds to a range of stimulus rates, (2) to determine whether the fetal auditory brain stem response exhibits latency changes with increasing stimulus rates, and if so, (3) to determine the influence of fetal age on the latency response of the auditory brain stem response to increasing stimulus rates. The results of this study provide new information on the development of auditory function in the fetus during the final weeks of pregnancy and define critical periods of auditory development, during which time adverse perinatal conditions may contribute to congenital hearing loss.

Methods

Fetal instrumentation. Eight fetal lambs (three from twin pregnancies and five singletons) were instrumented at 110 to 118 days' gestation (term = 145 days) for measurement of fetal auditory brain stem responses. After each pregnant ewe was administered spinal block anesthetic (tetracaine hydrochloride), a sterile midline abdominal incision was made to expose

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the uterus. The left side of the fetal neck was marsupialized through a small uterine incision for catheterization of the fetal carotid artery. All fetal surgery was conducted after local injection of subdermal anesthetic (lidocaine [Xylocaine, 2%]). A catheter was placed into the lumen of the fetal carotid artery and passed to the level of the ascending aorta. After the neck incision was closed, the fetal head was maneuvered to the uterine incision. Stainless steel wires insulated with Teflon and encased in polyvinyl tubing were secured subdermally at the vertex and anterior to the right pinnae of the fetal head. These wires were used as the active and reference electroencephalographic electrodes, respectively. A Danavox Model CN (68 ohm) hearing aid receiver, which was covered with a condom, was secured in the fetal external ear canal ipsilateral to the reference electrode and contralateral to the side of the fetal carotid artery catheter. After fetal instrumentation was complete, the fetal head was returned to the uterus and the uterine and abdominal incisions were closed. The earphone leads, stainless steel wires, and arterial catheter were brought out the abdominal incision and passed through a subdermal tunnel to a pouch on the flank of the ewe.

Auditory brain stem response testing. Fetal auditory brain stem responses were obtained in response to a series of clicks produced by the Danavox earphone secured in the fetal ear. Each click represented a square wave pulse of 0.1 msec duration and of alternating pulse polarity. The stimulus intensity used in this study was 105 dB sound pressure level when measured in a 6 cc coupler of a sound level meter (Model 452, Scotts Instruments Laboratory) in the fast mode and with the linear scale.

The responses from 1200-click stimuli were amplified 10^6 times and averaged by a Synap I signal averaging computer (Infant Hearing Assessment Foundation, San Francisco, Calif.) with a 200 to 2500 Hz bandpass and a 50 μsec sampling interval. The final averaged response was plotted on heat-sensitive paper with a 10 msec grid. All auditory brain stem response tests were conducted in duplicate to ensure reproducibility between recordings. A wave deflection was considered reproducible when the latency varied by no more than 0.2 msec between auditory brain stem response recordings at the same click rate.

Testing for fetal auditory brain stem responses was begun the day after surgery. If auditory brain stem responses were obtained at the initial click rate of 16 clicks/sec, the auditory brain stem response tests were repeated at increasing click rates of 32, 45, and 64 clicks/sec to determine the maximal click rate that would elicit an auditory brain stem response. If fetal auditory brain stem responses were reproducible up to 64 clicks/sec, tests at 87 and 100 clicks/sec were also conducted. The animals were randomly retested at intervals of 1 to 8 days. Twice weekly, blood samples (0.5 ml) were withdrawn from the fetal arterial catheter for blood gas determination by a 1.0 ml heparinized glass syringe. The fetal arterial sample was analyzed for pH, P_{CO_2}, and P_{O_2} levels with a BMS-3 Radiometer blood gas analyzer adjusted to 39° C (normal sheep core temperature).

Qualitative and quantitative analyses were conducted on the data as follows: The reproducible auditory brain stem response to the highest click rate was determined for each animal for each testing day. This qualitative determination was conducted by visual inspection of the auditory brain stem response tracings for (1) reproducibility between individual tracings for each click rate and (2) similarity in wave pattern to auditory brain stem response tracings obtained at lower click rates. Linear regression was performed on this data to determine the rate of transition of responsiveness from slow click rates to fast click rates with increasing age.

Latency determinations were made only during testing sessions in which auditory brain stem response recordings were obtained at all six click rates (16, 32, 45, 87, and 100 clicks/sec). This approach was used to eliminate bias from latency measurements of auditory brain stem response of earlier age fetuses who exhibited auditory brain stem responses only at the lower click rates. The latency interval, in milliseconds, from the click stimulus to the first wave deflection (wave I) was determined as a measure of the peripheral auditory response. Latency measurements were also obtained from the click stimulus to wave V. The I-V interwave latency interval (central conduction time) was taken as a measure of the transmission time through the auditory tracts and nuclei within the brain stem and midbrain.

A two-way analysis of variance with repeated measures was performed on the latency data to determine age and click rate effects and the interaction effects on auditory brain stem response peak latencies and central conduction time values.

Results

All fetal lambs were healthy during the study. Analysis of fetal arterial blood samples for blood gas determinations was as follows: pH = 7.34 ± 0.11; P_{CO_2} = 34.5 ± 5.7 mm Hg; P_{O_2} = 23.0 ± 3.7 mm Hg (data presented as mean ± 1 standard deviation).

Auditory brain stem responses were initially recorded at 16 clicks/sec from all fetuses between 115 and 124 days' gestation. As previously reported from our laboratory, these initial auditory brain stem responses consisted of a five-wave complex of small reproducible deflections that increased in resolution as

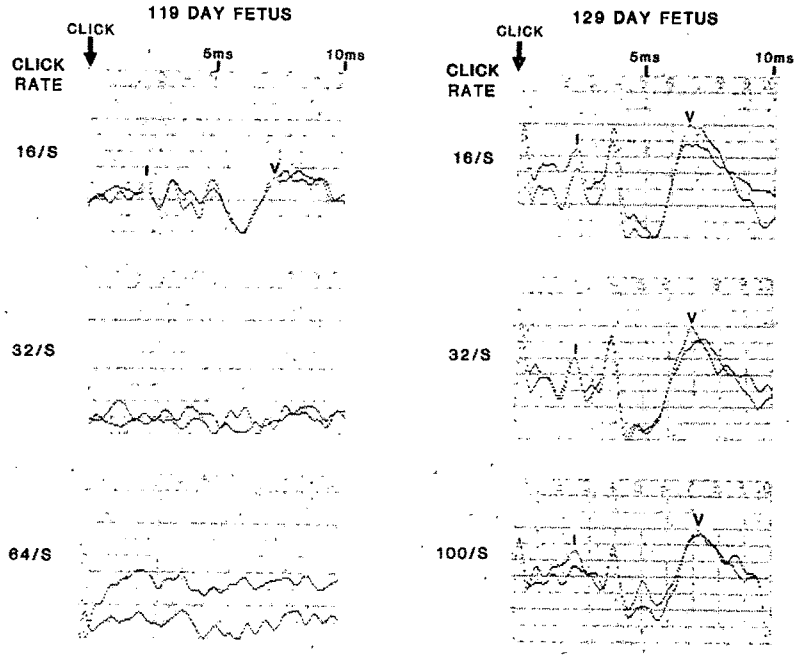


Fig. 1. Typical fetal auditory brain stem responses to increasing click rates at two different gestational ages.

the fetuses matured. Fig. 1 illustrates responses to click rates of 16, 32, and 64 clicks/sec from a 119-day fetus and responses to click rates of 16, 32, and 100 clicks/sec from a 129-day fetus. In this example, auditory brain stem responses were obtained at the lower click rate of 16 clicks/sec but not at 32 or 64 clicks/sec in the 119-day fetus. In the 129-day fetus, auditory brain stem responses were recorded up to 100 clicks/sec.

The extent of auditory brain stem response testing in all eight fetuses is graphically represented in Fig. 2. This figure indicates the gestational age of the fetal lambs at the time of surgery, the maximum click rate at which an auditory brain stem response could be obtained on a given day of testing, and the days of auditory brain stem response testing conducted on all eight fetuses. The lines connecting data points illustrate the progression of auditory brain stem response and click rate responsiveness in each individual fetus. Best-fit linear regression analysis of the progression of click rate responsiveness from 16 to 100 clicks/sec indicated a slope value of +14.2 click rate per gestational day. Once auditory brain stem responses were measured at 100 clicks/sec in each fetus, subsequent testing during the remainder of the testing period demonstrated continued auditory brain stem reponsiveness to all click rates up to 100 clicks/sec. Two ewes contracted pneumonia and died at 121 and 125 days. Fetal health in these two ewes as judged by the normal fetal blood gas and pH values was not compromised during the periods of fetal auditory brain stem response testing. The remaining six fetuses spontaneously delivered between 135 and 138 days' gestation, but died in the process of birth, presumably from the restraint of the fetal instrumentation.

Latency values for wave I, wave V, and the I-V interpeak values (central conduction time) are presented in Table I for each click rate at two gestational age groups of 117 to 126 and 129 to 136 days. All wave deflections used in the latency determinations were reproducible between auditory brain stem response tests, since none varied by any more than 0.2 msec at the same click rate. The data were divided into two groups to illustrate the effects of age and click rate on the auditory brain stem response latencies and central conduction time values. Comparisons of wave I, wave V, and central conduction time latency values in the two fetal age groups indicate decreasing latency values with increasing fetal age.

Two-way analysis of variance with repeated measures was conducted on the entire data set (not each age group separately). Two-way analysis of variance indicated significant effects of age on the latencies of wave I, wave V, and central conduction time values (wave I: $F = 393.6$, $df = 10$, $p < 0.001$; wave V: $F = 206.5$, $df = 10$, $p < 0.001$; central conduction time: $F = 45.3$, $df = 10$, $p < 0.001$). Two-way analysis of variance also indicated significant effects of click rate on the latencies of wave I, wave V, and central conduction time values (wave I: $F = 26.2$, $df = 5$,

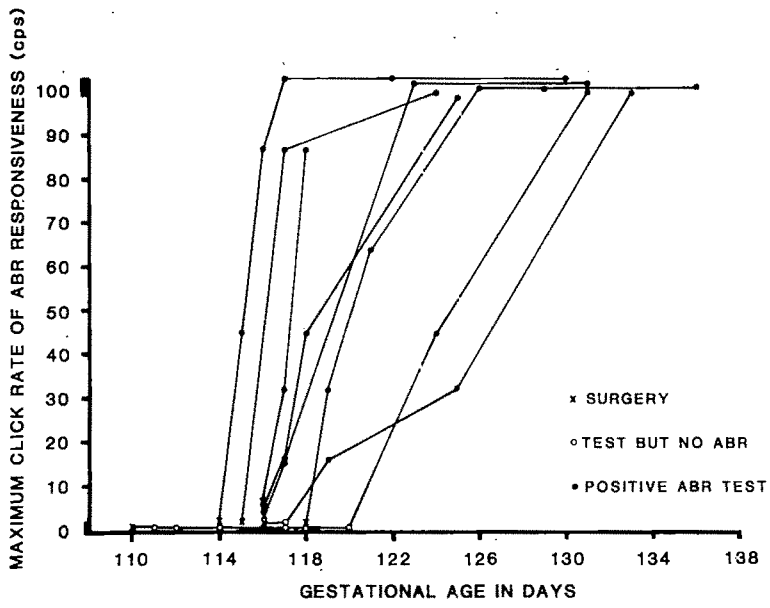


Fig. 2. The gestational age of surgery and the influence of advancing fetal age on maximal click rate responsiveness. The "x"s indicate the gestational age at the time of surgery. *Open circles* indicate days in which auditory brain stem response tests were conducted, but no auditory brain stem responses were obtained. *Solid circles* indicate the maximal click rate at which an auditory brain stem response could be obtained on the gestational age tested. (*cps* = Clicks per second; *ABR* = auditory brain stem response.)

Table I. Effect of increasing click rates on latency measurements of wave I, wave V, and CCT from auditory brain stem responses obtained from eight fetuses; data are presented as mean ± SEM; values in parentheses indicate number of tracings from which measurements were made

Click rate/sec	*Latencies (msec)*					
	Wave I		*Wave V*		*Central conduction time*	
	117-126 days	*129-136 days*	*117-126 days*	*129-136 days*	*117-126 days*	*129-136 days*
16	2.75 ± 0.09 (12)	2.29 ± 0.09 (10)	7.38 ± 0.17 (12)	6.71 ± 0.13 (10)	4.63 ± 0.12 (12)	4.45 ± 0.16 (10)
32	2.83 ± 0.11 (12)	2.34 ± 0.10 (10)	7.36 ± 0.18 (12)	6.72 ± 0.07 (10)	4.53 ± 0.11 (12)	4.38 ± 0.13 (10)
45	2.85 ± 0.10 (12)	2.41 ± 0.14 (10)	7.61 ± 0.18 (12)	6.77 ± 0.06 (10)	4.73 ± 0.12 (12)	4.36 ± 0.09 (10)
64	2.92 ± 0.11 (12)	2.48 ± 0.13 (10)	7.82 ± 0.23 (12)	6.85 ± 0.13 (10)	4.91 ± 0.13 (12)	4.37 ± 0.06 (10)
87	3.00 ± 0.09 (12)	2.42 ± 0.10 (10)	7.98 ± 0.17 (12)	6.78 ± 0.05 (10)	4.98 ± 0.10 (12)	4.50 ± 0.06 (10)
100	3.00 ± 0.07 (12)	2.35 ± 0.07 (10)	8.01 ± 0.12 (12)	7.11 ± 0.17 (10)	5.02 ± 0.07 (12)	4.76 ± 0.11 (10)

$p < 0.001$; wave V: $F = 45.5$, $df = 5$, $p < 0.001$; central conduction time: $F = 19.8$, $df = 5$, $p < 0.001$). The interaction of age and click rate from the two-way analysis of variance indicated significant interactions on wave I latency: $F = 7.4$, $df = 50$, $p < 0.001$; wave V latency: $F = 5.1$, $df = 50$, $p < 0.001$; and central conduction time values: $F = 4.7$, $df = 50$, $p < 0.001$.

Comment

In the present study, we determined changes in characteristics of the auditory brain stem response to different stimulus rates in the chronically instrumented fetal lamb. This objective was accomplished by measuring auditory brain stem responses in utero at click rates of 16, 32, 45, 64, 87, and 100 clicks/sec during the final 26 days of gestation. Results obtained from earlier age fetuses indicate that auditory brain stem responses are initially measurable only at slow click rates. Once these initial auditory brain stem responses appear, subsequent auditory brain stem responses in response to click rates up to 100 clicks/sec are measurable over the next 3 to 10 days.

Fetal auditory brain stem response latencies to wave I, wave V, and central conduction time in the present study decreased as a function of gestational age. Results of auditory brain stem response studies in in-

fants, children, and adults have also demonstrated latency changes in auditory brain stem response peaks because of age and rate of stimulus presentation. From infancy to early adulthood, auditory brain stem response latencies decrease with increasing age.[7-9] These latency decreases are thought to represent increased myelination and synaptogenesis occurring in the brain stem and midbrain.[7, 10, 11] Fetal auditory brain stem response latencies also increased with increasing rates of stimulus presentation. Similar findings have been reported in human newborns and adults.[8, 9, 12, 13] The effects of click rate on auditory brain stem response latencies have been attributed by Don et al.[12] primarily to receptor adaptation and fatigue. However, Pratt and Sohmer[13] and Pratt et al.[14] have suggested that the latency increase of the auditory brain stem response is caused by reduced synaptic efficacy; that each successive wave of the ABR is influenced by the preceding wave. As such, when the click rate is increased, a smaller number of incoming fibers are active and their synapses are less effective. As a result, it takes longer for the excitory synaptic potential to reach the firing threshold for the outgoing fibers and hence the longer auditory brain stem response latencies at the higher click rates. This hypothesis also suggests that each successive wave exhibits a greater change in latency with increasing click rate when compared with the preceding wave(s).

The presence of auditory brain stem responses at various click rates in this study appears to be an all-or-none phenomenon. From the tracings in which auditory brain stem responses were not measurable at the faster click rates (115- to 125-day fetuses), there were no reproducible peaks in these tracings (Fig. 1). The use of alternating polarity clicks presented at high stimulus rates of 80+ clicks/sec may sometimes result in complete cancellation of wave I. Click polarity (rarefaction, condensation, or alternating), however, has no effect on the presence of or differences in latency values of wave V.[15] Although alternating polarity clicks were used in the present study, wave I was easily identifiable and reproducible at the higher click rates when auditory brain stem responses were present (Fig. 1). The absence of wave V and all other preceding auditory brain stem response peaks in the younger fetuses at the higher click rates suggests that the other auditory brain stem response peaks are dependent on the ability of the peripheral auditory apparatus to recover from high stimulus rates to properly conduct impulses. Therefore maturation must occur in the peripheral auditory apparatus (cochlea) as the fetus becomes older to integrate incoming auditory signals into the brain stem more effectively. Similar observations have been reported in a study of premature human infants in which auditory brain stem responses were obtained at a range of stimulus rates. Despland and Galambos[9] reported one infant at 30 weeks who did not have a wave V response to stimulus rates above 40 clicks/sec. Within 2 weeks, however, wave V responses were obtained at rates as high as 80 clicks/sec.

The results of the present study indicate that rapid change in auditory click rate responsiveness occurs in the fetal lamb from 115 to 125 days' gestation. This period of rapid change may be indicative of a time window in which critical auditory changes occur. Since the immature nervous system is most vulnerable to toxic damage when growth is rapid,[16] the peripheral auditory system may be especially vulnerable during this period of development. Despland and Galambos[9] observed that the highest incidence of hearing impairments occurred in premature infants who were acidotic at birth. Therefore it is reasonable to speculate that toxic damage from an abnormal perinatal event such as acidosis during this period of rapid change in auditory function may potentiate the risk of hearing impairment. The fetal model presented in this study offers a unique opportunity to test a variety of perinatal conditions during specific periods of fetal auditory development.

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Pituitary responses to synthetic corticotropin-releasing hormone: Absence of modulatory effects by estrogen and progestin

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To determine if the activity of the adrenocorticotropic hormonal–adrenal axis is modulated in part by estrogens and progestins, we have compared pituitary and adrenal responses to corticotrophin-releasing hormone in normal women during the early follicular, late follicular, and midluteal phases of the menstrual cycle and in four women undergoing ovariectomy who received estradiol (E_2) implants alone or in combination with oral medroxyprogesterone acetate administration. Basal adrenocorticotropic hormone and cortisol levels (9:00 AM) and responses to ovine CRH were unaffected by variations in E_2 and progesterone during each phase of the menstrual cycle. In women undergoing ovariectomy who received E_2 replacement therapy, basal concentrations of adrenocorticotropic hormone and cortisol (9:00 AM) and the responses to human corticotrophin-releasing hormone were not altered by administration of E_2 alone or E_2 with medroxyprogesterone acetate. Under these experimental conditions, our findings suggest that physiologic changes in E_2 and progesterone levels during the menstrual cycle and replacement doses of estrogen and progestin commonly used clinically do not significantly influence basal adrenocorticotropic hormone and cortisol levels or responsiveness to corticotrophin-releasing hormone. (AM J OBSTET GYNECOL 1987;157:1387-91.)

Key words: Adrenocorticotropic hormone, corticotrophin-releasing hormone, medroxyprogesterone acetate, estrogen, progestin

Although it is widely recognized that there is an increase in the onset of depressive syndromes[1] and aberrant psychologic behavior[2] during the premenstrual phase of the menstrual cycle, the role of ovarian steroids in these disorders remains unclear. Since the pituitary-adrenal axis has been implicated as a major neuroendocrine modulator of psychologic behavior and stress responses,[3] preliminary studies have focused on peripheral measurements of adrenocorticotropic hormone (ACTH) and cortisol output. These studies suggest that in normal women, basal levels of ACTH and cortisol are elevated during the late follicular and midcycle phases of the menstrual cycle,[4] whereas in the presence of high progestin levels, basal cortisol concentrations have been reported to decrease.[5] On the other hand, other investigators have reported that there is an enhanced adrenocortical responsiveness to

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stress during the luteal phase of the cycle.[6] In view of these disparate findings, it seems necessary to examine first the modulatory role of ovarian steroids in ACTH-cortisol secretion at the pituitary and adrenal levels. To investigate these interactions, we have determined the pituitary and adrenal responses to corticotropin-releasing hormone in normal women during the menstrual cycle and in women underoing ovariectomy receiving hormone replacement therapy.

Material and methods

Six normal women between the ages of 26 and 43 years, with menstrual cycles between 28 and 32 days in length, volunteered for this study. Four women undergoing ovariectomy between the ages of 40 and 60 years were also studied. Each woman was within 10% of her ideal body weight.

In each experiment, subjects were admitted to the Clinical Research Center at 8:00 AM after an overnight fast. One hour after intravenous catheter placement, blood samples were collected at −20, 0, 20, 40, 60, 80, 100, 120, 150, and 180 minutes relative to the injection of corticotrophin-releasing hormone. Each woman with a normal cycle received either saline solution or ovine corticotrophin-releasing hormone, (1 μg/kg, intravenous bolus) during the early follicular phase (days 1 through 5) of the first menstrual cycle. During subsequent menstrual cycles, ovine corticotrophin-releasing hormone was administered during the late follicular (days 11 to 14) and midluteal (6 to 8 days after ovulation) phases. The timing of the late follicular and midluteal phase experiments was determined by pelvic ultrasound monitoring.

During the 2 years of these experiments, human corticotrophin-releasing hormone became available. Since both ovine corticotrophin-releasing hormone and human corticotrophin-releasing hormone have similar biologic action,[7] our laboratory switched exclusively to human corticotrophin-releasing hormone. Thus this latter neuropeptide was used in our experiments in women undergoing ovariectomy. Each woman undergoing ovariectomy received a subcutaneous 25 mg estradiol (E_2) pellet for estrogen replacement. This dose of E_2 maintains constant E_2 levels in the physiologic range (80 to 120 pg/ml).[8] Two to 3 months after E_2 replacement, provocative testing with human corticotrophin-releasing hormone (1 μg/kg, intravenous bolus) was performed. Medroxyprogesterone acetate, 10 mg by mouth, was then administered for 10 days. On the tenth day of medroxyprogesterone acetate administration, provocative testing with human corticotrophin-releasing hormone was repeated. During these experiments no discernible side effects were noted. This project was approved by the Human Subjects Committee of the University of California, San Diego.

Synthetic ovine corticotrophin-releasing hormone and human corticotrophin-releasing hormone were prepared in an identical fashion. Each peptide was dissolved in 5% mannitol (w/v) with 10^{-4}mol/L of ascorbic acid and sterile water at a final concentration of 200 μg/ml. The solution was then sterilized by millipore filtration, lyophilized, and stored in siliconized vials at −70° C. Corticotropin-releasing factor was reconstituted with sterile water immediately before each experiment.[9] Blood samples for ACTH were collected in 2 ml polypropylene tubes containing ethylenediaminetetraacetic acid (Sarstedt, West Germany) and promptly centrifuged at 4° C. Plasma was snap frozen in dry ice/ethanol within 10 minutes after collection and stored at −70° C until assayed. Concomitant serum samples were obtained for luteinizing hormone (LH), follicle-stimulating hormone (FSH), E_2, progesterone 4, and cortisol measurements.

ACTH was determined in unextracted plasma by radioimmunoassay in duplicate.[10] This assay uses high-performance liquid chromatography purified iodine 125–$ACTH_{1-39}$ tracer (Radioassay Systems Laboratory, Inc., Carson, Calif.) and a commercially available antiserum from the IgG Corp. (Nashville, Tennessee). Serum cortisol, LH, FSH, E_2, and progesterone concentrations were measured by previously established radioimmunoassays.[9, 11] All samples from each subject were measured in a single assay. The intraassay coefficient of variation for ACTH was 2.4% to 5.8% at standard doses between 3.3 and 34.6 pg/ml. The range of intraassay coefficient of variation for cortisol was 2.8% to 6.2% between standard doses of 40.3 and 394 ng/ml. Interassay coefficients of variation were 11.8% and 6.5% for ACTH and cortisol, respectively.

ACTH and cortisol responses were analyzed by a three-factor analysis of variance with Newman-Keuls testing to examine for potential differences in responses to corticotrophin-releasing hormone for each phase of the menstrual cycle. The integrated response curves were calculated by the trapezoid method and analyzed by the Student t test. All data are expressed as mean ± SE.

Results

Mean basal levels of LH, FSH, E_2, and progesterone during each experiment are summarized in Table I. The LH, FSH, E_2, and progesterone concentrations during each phase of the menstrual cycle are consistent with previously reported values.[12] In women undergoing ovariectomies, LH and FSH levels were elevated in comparison with normal women, although E_2 concentrations were maintained in the physiologic

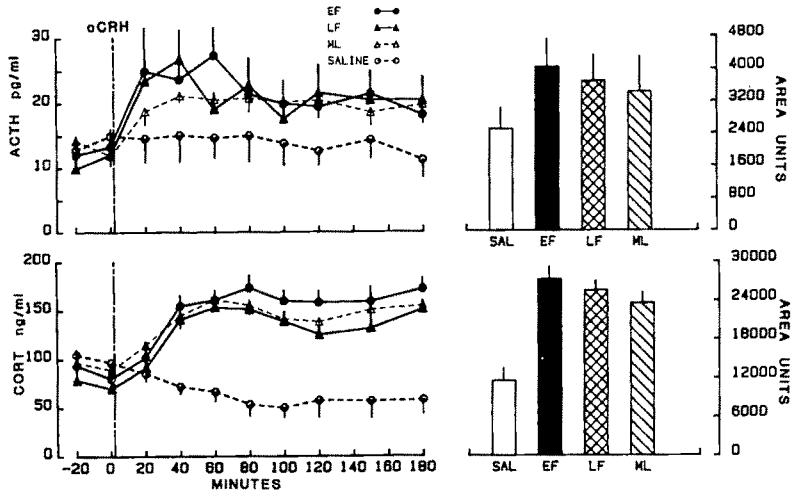


Fig. 1. *Left,* Mean (± SEM) ACTH and cortisol concentrations after the administration of saline solution or ovine corticotrophin-releasing hormone (1 μg/kg, intravenous bolus) in six women during the early follicular, late follicular, and midluteal phases of the menstrual cycle. *Right,* Integrated ACTH and cortisol responses (± SEM) to ovine corticotrophin-releasing hormone (1 μg/kg, intravenously) during the menstrual cycle.

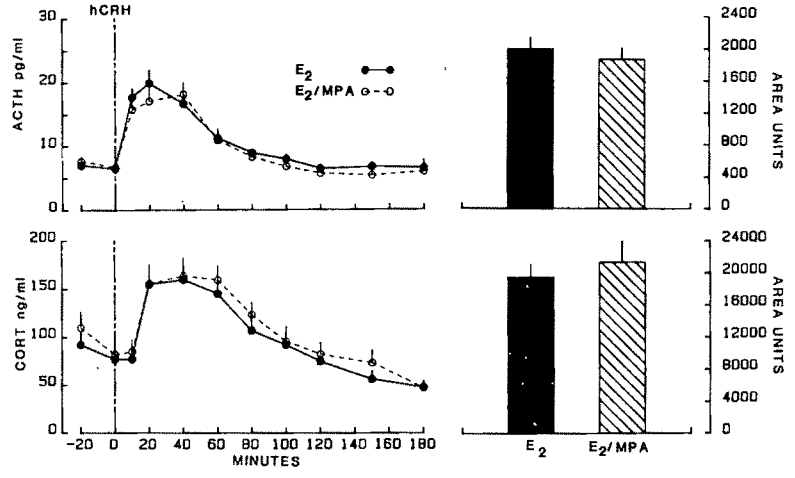


Fig. 2. *Left,* Mean (± SEM) ACTH and cortisol concentrations after human corticotrophin-releasing hormone administration (1 μg/kg, intravenous bolus) in four women undergoing ovariectomy receiving subcutaneous E_2 pellets or E_2 pellets and medroxyprogesterone acetate. *Right,* Integrated ACTH and cortisol responses (± SEM) to human corticotrophin-releasing hormone (1 μg/kg, intravenously) during E_2 and E_2 with medroxyprogesterone acetate.

range. Despite the variations in E_2 and progestin levels during each experiment, basal ACTH and cortisol concentrations were not significantly different within each group. Although basal ACTH levels were not different between the two groups, basal cortisol levels were elevated ($p < 0.01$) in women undergoing ovariectomy receiving E_2 and medroxyprogesterone acetate when compared with normal women in the late follicular phase.

During saline solution administration in normal women, ACTH levels remained stable while cortisol concentrations exhibited its characteristic midmorning decline (Fig. 1). The administration of ovine corticotrophin-releasing hormone elicited a prompt release of ACTH that reached a peak increment at 40 minutes. Peak ACTH levels were 27.3 ± 4.2, 26.3 ± 4.4, and 20.7 ± 1.0 pg/ml for the early follicular, late follicular, and midluteal phases, respectively. ACTH levels then declined but remained elevated above baseline levels during the next 2 hours. Analysis of the ACTH response curves and the integrated response for each phase of the menstrual cycle did not demonstrate

Table I. Mean (±SEM) basal hormone levels in women with normal cycles and ovariectomies

	Normal women (n = 6)			*Ovariectomized women (n = 4)*	
	EF	*LF*	*ML*	*E_2 pellet*	*E_2 + MPA*
ACTH (pg/ml)	13.3 ± 2.7	11.7 ± 2.9	11.5 ± 1.6	7.0 ± 0.5	7.7 ± 0.8
Cortisol (ng/ml)	80 ± 14	70 ± 7	89 ± 14	81 ± 13	121 ± 10*
LH (mIU/ml)	10.3 ± 0.5	19.7 ± 4.5	6.7 ± 0.8	69.6 ± 36.5	38 ± 16.3
FSH (mIU/ml)	16.4 ± 0.9	15.9 ± 2	8.4 ± 0.7	45.3 ± 22.5	41 ± 19.5
E_2 (pg/ml)	48 ± 7	265 ± 82	223 ± 83	139 ± 48	132 ± 40
Progesterone (ng/ml)	0.4 ± 0.1	1.2 ± 0.3	12.1 ± 2.6	—	—

EF = Early follicular phase; LF = late follicular phase; ML = midluteal phase; MPA = medroxyprogesterone acetate.
*$p < 0.01$ versus late follicular phase.

significant differences. Cortisol responses to ovine corticotrophin-releasing hormone during each phase of the cycle were parallel to ACTH, with peak increments observed at 80 minutes (Fig. 1). No significant differences in either peak or integrated cortisol responses were evident between different phases of the menstrual cycle.

In women undergoing ovariectomy receiving E_2 replacement, human corticotrophin-releasing hormone administration elicited a more rapid pituitary release of ACTH and achieved peak responses (19.9 ± 2.1 pg/ml) by 20 minutes (Fig. 2). However, the duration of ACTH release after administration of human corticotrophin-releasing hormone was much shorter than that of ovine corticotrophin-releasing hormone, with ACTH levels returning to basal levels by 100 minutes. These differences are compatible with previous reports[7] and probably reflect the longer half-life of ovine corticotrophin-releasing hormone[13] rather than intrinsic differences in the pituitary-adrenal axis between the two groups. Serum cortisol concentrations increased 10 to 20 minutes after human corticotrophin-releasing hormone and were parallel to ACTH (Fig. 2). The combination of E_2 and medroxyprogesterone acetate treatment did not affect ACTH and cortisol responses to human corticotrophin-releasing hormone.

Comment

To demonstrate the potential modulatory effects of gonadal steroids, we selected a dose of ovine corticotrophin-releasing hormone (1 μg/kg), which represented a half-maximal stimulus.[13] Similarly, the dose of human corticotrophin-releasing hormone (1 μg/kg) used in this study has been reported to be submaximal.[7] Under these experimental conditions, we have demonstrated that pituitary and adrenal responses to ovine corticotrophin-releasing hormone in women with normal cycles were not altered by physiologic fluctuations in ovarian steroid concentrations during different phases of the menstrual cycle. In addition, ACTH and cortisol responses to human corticotrophin-releasing hormone women having ovariectomy were similar during E_2 or E_2 with medroxyprogesterone acetate administration. To the best of our knowledge, this is the first study that has examined the potential modulatory role of gonadal steroids on pituitary and adrenal responses to corticotrophin-releasing hormone.

Basal ACTH and cortisol levels during each phase of the menstrual cycle were also not significantly different. These results are in agreement with Aubert et al.,[11] but are at variance with findings reported by Genazzani et al., who identified midcycle elevations in ACTH and cortisol during daily morning determinations. Since ACTH and cortisol both display accentuated episodic secretion during the morning hours,[15] this pulsatile pattern could account for the reported differences in basal hormone levels in these studies and the increased cortisol levels observed in women undergoing ovariectomy receiving E_2 and medroxyprogesterone acetate in our study.

Our present results differ from previous reports in which pharmacologic administration of estrogens and progestins appeared to exert opposite influences on the pituitary-adrenal axis. Estrogen administration has been reported to increase peripheral cortisol levels,[16] which probably reflects in part an estrogen-mediated increase in the hepatic production of corticosteroid-binding globulin.[17] In contrast, progestins appear to suppress the pituitary-adrenal axis by virtue of its weak glucocorticoid properties.[18] Both glucocorticoid and progesterone receptor complexes have been reported to share binding sites to the same regulatory region of deoxyribonucleic acid.[19] When administered in pharmacologic doses, progestins effectively inhibit corticotrophin-releasing hormone–mediated ACTH release in pituitary cell cultures,[20] inhibit ACTH synthesis and release, and can cause adrenal atrophy.[21] In our present study, however, the administration of smaller replacement doses of E_2 and medroxyprogesterone acetate in women undergoing ovariectomy had relatively little effect on basal ACTH and cortisol le-

vels or pituitary responses to corticotrophin-releasing hormone.

In summary, a comparison of the pituitary and adrenal activity under different gonadal steroid environments in women failed to demonstrate significant effects during basal or corticotrophin-releasing hormone–stimulated conditions. In view of recent reports of altered responses to corticotrophin-releasing hormone in various depressive disorders,[22] these findings would indirectly support the notion that variations in psychologic behavior and stress responses premenstrually reflect changes mediated primarily at a suprapituitary level.

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Effect of pregnancy on surgically induced endometriosis in cynomolgus monkeys

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There are few objective data to support the clinical impression that pregnancy exerts a beneficial effect on endometriosis. In this study, we examined the progression-regression of endometriosis in pregnant and nonpregnant monkeys. Endometriosis was surgically induced in 16 monkeys and classified as minimal (n = 5), mild (n = 5), moderate (n = 4), or severe (n = 2). Monkeys were mated and pregnancy occurred in three monkeys with minimal, three with mild, and one with moderate endometriosis. Laparotomy was performed to stage the disease and to obtain biopsy specimens of implants 1 month post partum or 6 to 8 months after initial operation in nonpregnant monkeys. Monkeys with minimal and mild disease had complete regression of macroscopic disease. Histologic examination of the implants showed only fibrosis and hemosiderin deposits, except in one animal with mild endometriosis containing microscopic foci of endometrial glands. The one monkey with moderate disease had significant, but not complete regression of implants. Endometriosis in nonpregnant monkeys tended to progress. These findings demonstrate that pregnancy does exert a beneficial effect on endometriosis and suggest that pregnancy commonly results in complete resolution of minimal or mild disease. (AM J OBSTET GYNECOL 1987;157:1392-6.)

Key words: Endometriosis, pregnancy, resolution

The simultaneous occurrence of endometriosis and pregnancy is well established.[1-3] Although endometriosis may become symptomatic during pregnancy, early reports suggested that pregnancy exerts a beneficial effect on endometriosis.[4] McArthur and Ulfelder[5] critically analyzed these cases and concluded that the behavior of endometriosis during pregnancy is extremely variable. Pregnancy was followed by a reduction in size of implants in most cases, but permanent regression of endometriosis was encountered less frequently than persistent disease.

There are few objective data documenting the macroscopic and microscopic changes in endometriosis as the result of pregnancy. In conducting previously reported studies on the causes of infertility in monkeys with endometriosis, we had the opportunity to critically assess the effect of term pregnancy on surgically induced endometriosis.[6] This report describes our findings and confirms the clinical impression that pregnancy has a beneficial effect on endometriosis.

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Material and methods

Twenty-one adult female cynomolgus monkeys *(Macacca fascicularis)* demonstrating menstrual cycle regularity were selected for study. Housing, feeding, and general husbandry have been described previously.[6]

Endometrial (n = 11) or adipose (n = 10) tissue was autografted to the pelvic peritoneum 3 to 5 days after the midcycle 17β-estradiol peak, as previously described.[6] Briefly, a hysterotomy was performed and resected endometrial tissue was minced and injected subperitoneally. In controls, endometrial tissue was discarded and adipose tissue injected. Laparotomy was performed during the next menstrual cycle to stage the disease and obtain biopsy specimens of the implants for histologic study.[7]

Monkeys classified as having minimal, mild, and moderate endometriosis developed peritoneal implants only. Peritoneal implants appeared as well-vascularized reddish nodules or "powder burn" deposits and ranged from 1 to 4 mm in size. Histologic examination of these areas demonstrated viable endometrial glands and stroma and hemosiderin deposits (Figs. 1 and 2). Monkeys with severe disease developed cystic adnexal masses that contained hemorrhagic, thick fluid ("chocolate cysts"). Pelvic adhesions were present only in monkeys classified as having moderate or severe disease. Biopsy of implants did not affect the macroscopic appearance or staging noted at subsequent laparotomy.



Fig. 1. Photomicrograph of biopsy specimen taken from a reddish blue peritoneal nodule. Note presence of viable endometrial glands and stroma *(arrow)*. (Hematoxylin and eosin. Original magnification ×150.)

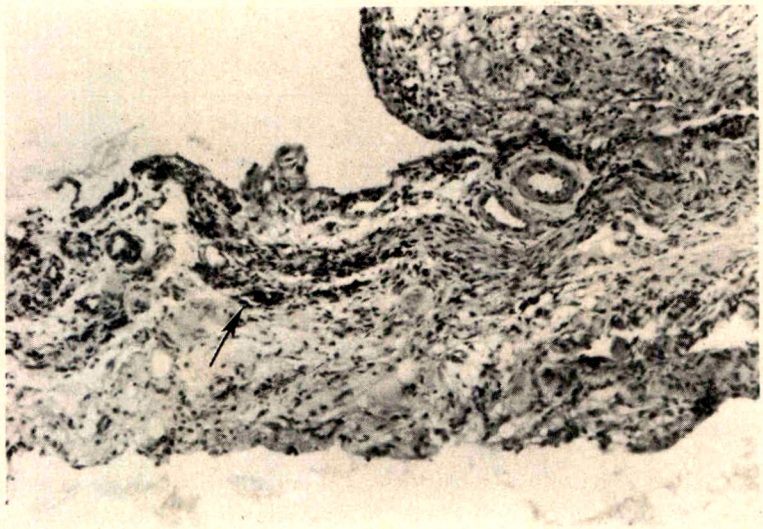


Fig. 2. Photomicrograph of biopsy specimen taken from a "powder-burn" lesion on peritoneum. Note presence of hemosiderin deposits and fragments of endometrial glands *(arrow)*. (Hematoxylin and eosin. Original magnification ×150.)

Five monkeys with adipose implants were found to have microscopic endometriosis and were classified as having minimal disease. Monkeys with endometrial autografts were classified as having mild (n = 5), moderate (n = 4), and severe (n = 2) disease (according to the American Fertility Society revised classification of endometriosis, modified to correct for the smaller size of the pelvic organs in monkeys).

During three subsequent menstrual cycles, female monkeys were mated with male animals of verified fertility. Laparotomy was performed after each mating and the extent of endometriosis and pelvic adhesions recorded. Three monkeys with minimal, three with mild, and one with moderate endometriosis delivered at term.

Laparotomy to stage endometriosis was performed 1 month post partum in pregnant monkeys and 6 to 8 months after surgical induction of endometriosis in nonpregnant monkeys. Sites of active or resolved endometriosis were sampled or resected, respectively. Specimens were serially sectioned and examined histologically. American Fertility Society classification

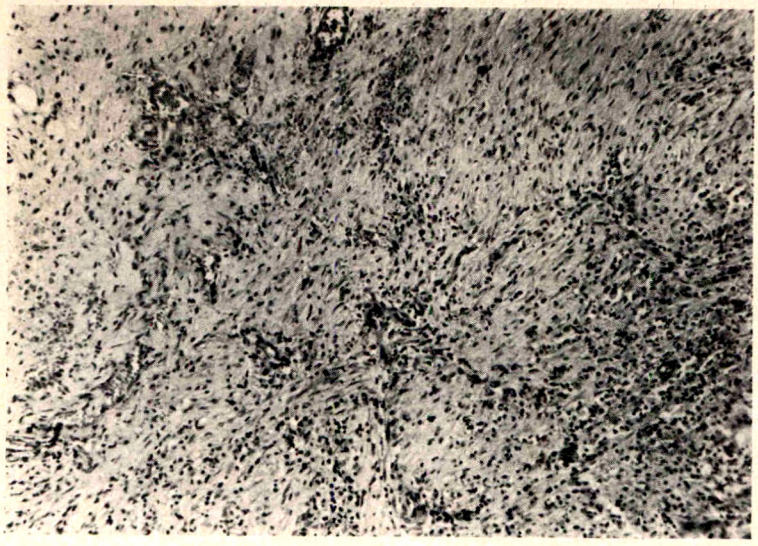


Fig. 3. Photomicrograph of biopsy specimen taken from fibrotic area of peritoneum 1 month after delivery. The biopsy site previously contained a 2 mm superficial raised reddish implant of histologically confirmed endometriosis. (Hematoxylin and eosin. Original magnification ×150.)

Table I. American Fertility Society revised classification of endometriosis in pregnant and nonpregnant monkeys

Initial American Fertility Society classification	*Score**	*n*	*American Fertility Society classification score**			
			Pregnant		*Not pregnant*	
			Initial	*Postpartum*	*Initial*	*Follow-up*
Minimal	1.6 ± 0.3	5	1.3 ± 0.4	0	2.0 ± 0.0	2.0 ± 0.0
Mild	7.6 ± 0.8	5	7.3 ± 0.7	3.0 ± 1.5	8.0 ± 2.0	9.0 ± 3.0
Moderate	20.5 ± 1.5	4	22	13	20.0 ± 2.3	22.7 ± 3.5
Severe	55.0 ± 1.9	2	—	—	55.0 ± 3.0	62.0 ± 2.0
	14.9 ± 4.4	16	6.9 ± 2.8	3.3 ± 1.8†	21.1 ± 6.9	23.8 ± 7.8

*Values expressed as mean ± SE.
†$p < 0.05$ versus initial American Fertility Society score.

scores from the first laparotomy after induction of endometriosis and from the last laparotomy were compared by paired *t* tests.

Results

The American Fertility Society classification scores at the initial staging laparotomy and at the postpartum or follow-up laparotomy for pregnant and nonpregnant monkeys in each classification group are shown in Table I. Monkeys with minimal and mild disease that delivered at term had complete regression of macroscopic endometriosis. Peritoneal biopsy specimens from sites previously afflicted with endometriosis showed only fibrosis and hemosiderin deposits (Fig. 3), except in one monkey with mild endometriosis found to have foci of endometrial glands. The monkey with moderate disease demonstrated marked but not complete regression of endometriotic implants and there was no change in the severity of pelvic adhesions. A significant decrease in American Fertility Society classification score was found after pregnancy ($p < 0.05$).

In contrast, monkeys that did not become pregnant had an overall progression of disease. Endometrial implants increased in size and, in particular, pelvic adhesions increased in severity. The latter was primarily responsible for the increase in the American Fertility Society classification score in nonpregnant monkeys.

Comment

In this study, endometriotic implants demonstrated complete resolution or marked reduction in size after term pregnancy. The beneficial effect of pregnancy on endometriosis was evidenced by a significant decrease in the American Fertility Society classification score.

The lower score was due exclusively to regression of endometriotic lesions, inasmuch as no change in severity of pelvic adhesion was noted at postpartum laparotomy. Endometriotic implants did not regress in nonpregnant monkeys, indicating that regression of disease in pregnant monkeys was due to pregnancy-related factor(s) and not to spontaneous resolution.

The observed beneficial effect of pregnancy on endometriosis is consistent with previous clinical and experimental studies. In McArthur and Ulfelder's review[5] of 23 cases, endometriotic lesions tended to enlarge during the first trimester and regress as pregnancy progressed. Complete resolution of disease after pregnancy occurred infrequently. Vernon et al.[8] also reported a suppressive influence of pregnancy on the size of autotransplanted "uterine squares" (whole sections of myometrium and endometrium) in rats. Implants were not visible during pregnancy but were found on microscopic examination to contain endometrial components. Implants were again grossly detected after the peripartum surge in 17β-estradiol. The authors concluded that pregnancy temporarily suppresses endometriosis but may not cure it. In our study, complete resolution of implants did occur, but only in monkeys with minimal or mild peritoneal disease.

The mechanism(s) whereby pregnancy suppresses endometriosis is unclear. In the rat model, pseudopregnancy, in animals with or without a uterus, results in suppression of endometriotic implants.[9] These observations suggest that an extrauterine, extraembryonic pregnancy factor or factors suppress endometriosis. During pregnancy, endometriotic implants generally show stromal decidualization and flattening of glandular epithelium.[5] Prolongation of this progestational effect of pregnancy may result in stromal edema and necrosis with resultant hyalinization, fibrosis, and atrophy of implants. These changes have been observed in endometriotic implants of women and monkeys treated with high-dose progestins.[10, 11] In contrast to our findings, hormonal "pseudopregnancy" has not resulted in a cure with complete fibrosis of the endometriotic implants.[10, 11]

The variable behavior of endometriosis during pregnancy suggests that factors in addition to the gravid hormonal milieu are regulating growth and maintenance of endometriotic tissue. Recent studies have demonstrated that steroid receptor concentrations in endometriotic implants are lower than those in eutopic endometrium and up to 20% of implants do not have measurable concentrations of estrogen or progesterone receptors.[12, 14] The differences in steroid receptor concentrations are consistent with histologic and ultrastructural studies that demonstrate an incomplete morphologic response of endometriotic implants to cyclic hormonal changes.[15, 16] The favorable effect of pregnancy or progestin therapy on endometriotic implants may be restricted to implants with progesterone receptors. Surgically induced endometriotic implants may have greater progesterone receptor concentrations than do spontaneous implants and thus respond more readily to the hormonal milieu of pregnancy.

In summary, pregnancy results in significant regression of surgically induced endometriosis in cynomolgus monkeys. Fibrosis and complete resolution of implants did occur, but only in monkeys with minimal or mild peritoneal disease. These findings demonstrate that pregnancy exerts a beneficial effect on endometriosis and suggest that pregnancy commonly results in complete resolution of minimal and mild peritoneal disease.

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Follicle-stimulating hormone is the main determinant of follicular recruitment and development in ovulation induction with human menopausal gonadotropin

Michael Vermesh, M.D., and Oscar A. Kletzky, M.D.
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The early hormonal changes that lead to follicular maturation and/or hyperstimulation in women requiring ovulation induction with either human menopausal gonadotropin or gonadotropin-releasing hormone have not been elucidated. This study was undertaken to assess the relative contribution of follicle-stimulating hormone and luteinizing hormone to estradiol secretion and follicular maturation in patients receiving human menopausal gonadotropin or gonadotropin-releasing hormone. The study group consisted of 10 women (26 to 38 years of age) with secondary amenorrhea as a result of hypothalamic dysfunction who had failed to ovulate when given clomiphene citrate. The patients were randomly assigned to either human menopausal gonadotropin ($n = 5$) or gonadotropin-releasing hormone ($n = 5$) treatment. On day 5 after the onset of induced menses, all women had baseline blood samples obtained at 10-minute intervals for4 hours. At this time either 150 U of human menopausal gonadotropin or 75 ng/kg of gonadotropin-releasing hormone administered hourly was given, and blood sampling every 10 minutes was continued for an additional 6 hours. Thereafter, patients were evaluated daily until ovulation. A significant and sustained increase in the mean plasma follicle-stimulating hormone level was first measured during the third hour after human menopausal gonadotropin administration ($p < 0.05$). The area under the curve of the mean plasma follicle-stimulating hormone value after this initial increase was significantly greater than its baseline (2119 ± 240 versus 1425 ± 188 mIU/ml; $p < 0.01$). This rise in mean follicle-stimulating hormone level was followed in less than 2 hours by a significant and uniform rise in mean plasma estradiol concentration ($p < 0.05$). In contrast, no immediate change in the mean levels of luteinizing hormone, follicle-stimulating hormone, or estradiol occurred after gonadotropin-releasing hormone administration. The mean daily levels of luteinizing hormone were similar in both groups; however, mean daily follicle-stimulating hormone (20.0 ± 1.1 versus 9.2 ± 1.4 mIU/ml) and estradiol (1004 ± 174 versus 495 ± 83 pg/ml) levels were significantly higher in patients treated with human menopausal gonadotropin than in those treated with gonadotropin-releasing hormone ($p < 0.001$ and $p < 0.05$, respectively). In addition, only in patients receiving human menopausal gonadotropin was a positive correlation found between mean daily plasma estradiol and follicle-stimulating hormone ($r = 0.685$, $p < 0.05$) levels and between mean daily plasma estradiol and prolactin ($r = 0.94$, $p < 0.001$) levels. In conclusion, this study suggests that follicle-stimulating hormone is the main factor involved in multiple ovarian follicular development and possible multiple gestation in patients treated with human menopausal gonadotropin. (Am J Obstet Gynecol 1987;157:1397-402.)

Key words: Human menopausal gondadotropin, follicle-stimulating hormone, ovulation induction, follicular maturation

Induction of ovulation in patients with hypothalamic amenorrhea who have failure of clomiphene citrate treatment is usually accomplished by the use of either human menopausal gonadotropin (menotropins, hMG) or gonadotropin-releasing hormone (GnRH).[1-4] Although hMG has been used extensively for many years, the mechanisms involving the initial ovarian responsivity have not been completely studied. Since both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) receptors were found to be present in the follicle, it is unclear whether the LH or FSH component of hMG has greater relevance in initiating and maintaining follicular stimulation.[5] Berger and Taymor[6] reported that high doses of human pituitary FSH were ineffective in stimulating follicular estrogen secretion in patients with hypothalamic amenorrhea and concluded that LH was essential for the induction of ovulation.[6] Others have demonstrated that

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the administration of human urinary FSH alone is sufficient to induce follicular growth and maturation in the presence of normal levels of endogenous LH.[7] diZerega and Hodgen[8] have suggested that in monkeys FSH is not the main determinant in establishing the final number of follicles which will ultimately ovulate.

It has been suggested that GnRH given in a pulsatile manner (every 60 to 120 minutes) induces frequent pulses of LH that reproduce the normal LH pulsatility leading to a single ovulation and pregnancy.[4, 9] However, mild ovarian hyperstimulation and multiple gestations have been reported with doses of ≥100 ng/kg/hr of GnRH, whereas 25 ng/kg/hr did not induce ovulation in all women.[4, 10]

The purpose of this study was to assess, from the inception, the hormonal dynamics leading to follicular recruitment, maturation, and ovulation in patients with hypothalamic amenorrhea treated with hMG and GnRH. The findings of these initial changes may help to elucidate the nature of multiple ovarian follicular stimulation and possible multiple gestation.

Material and methods

The study group consisted of 10 women (26 to 38 years of age) with secondary amenorrhea as a result of hypothalamic dysfunction of more than 6 months' duration, who had failed to ovulate with clomiphene citrate in doses of up to 250 mg daily for 5 days. All had a normal physical examination, were within the twentieth percentile of ideal body weight, and had no history of intensive exercise. None of these women had physical signs or laboratory test results consistent with polycystic ovarian disease. The patients were randomly assigned to either hMG (n = 5) or GnRH (n = 5) treatment. Informed consent was obtained from each individual before participation.

On day 5 after the onset of menses, induced with oral medroxyprogesterone acetate, all subjects were admitted to the Clinical Research Center and kept supine throughout the study. An indwelling catheter was inserted in an antecubital arm vein and kept open with a heparin lock. Baseline venous blood samples were obtained through the catheter at 10-minute intervals for 4 hours. At this time either 150 U of hMG (Pergonal, Serono, Randolph, Massachusetts) was given intramuscularly or 75 ng/kg of GnRH (Bachem, Carson, California) was initiated intravenously and administered hourly. Thereafter, blood sampling every 10 minutes was continued for an additional 6 hours. Patients were then discharged and followed up daily on an outpatient basis.

Those receiving hMG had the daily dose adjusted according to the levels of serum estradiol and the hMG was administered between 4:00 and 6:00 PM. The dose was increased by 50% if the serum estradiol did not double in 3 days. Each patient received a mean of 217 U or 2.9 ampules of hMG daily. Daily transvaginal ultrasound examination was performed when the serum estradiol level reached 200 pg/ml. When one or more follicles reached 17 to 19 mm in diameter a single dose of 5000 U of human chorionic gonadotropin was given intramuscularly to induce ovulation. Those patients receiving GnRH had the solution prepared with 1000 U heparin and saline solution up to a total volume of 5 ml each time refilling was required. GnRH was administered intravenously through a 4-inch-long, 19-gauge plastic catheter connected to a portable pulsatile infusion pump (Auto-Syringe, Travenol, Hooksett, New Hampshire). GnRH was delivered automatically every 60 minutes in constant doses of 75 ng/kg. This dose was expected to consistently induce ovulation without hyperstimulation.[8] With very few exceptions blood samples were obtained immediately before the hourly dose of GnRH.

All blood samples were collected between 8:00 and 9:00 AM in heparinized tubes and the plasma was separated in a refrigerated centrifuge within 1 hour and stored at −20° C until assayed. Plasma levels of LH, FSH, estradiol, and prolactin were determined by radioimmunoassay using previously described methods.[11-13] The sensitivity and the intra-assay and inter-assay coefficients of variation of the assays, respectively, were as follows: LH, 1.5 mIU/ml, 8.0% and 11.3%; FSH, 1.5 mIU/ml, 6.3% and 9.0%; estradiol, 10 pg/ml, 8.0% and 10.5%; prolactin, 2 ng/ml, 7.0% and 7.8%.

Baseline LH, FSH, estradiol, and prolactin were calculated by averaging all values obtained during the first 4 hours. Statistical analysis of frequent measurements of all hormones for the 4-hour baseline and the six initial hours after the commencement of treatment was performed by analysis of variance. In those instances in which significant changes were obtained by analysis of variance, the approximate time interval of a significant rise from the initiation of drug administration was determined by paired t test, with the Bonferroni correction for multiple comparisons with an overall significance level of $p < 0.05$.[14] To evaluate the degree of LH, FSH, estradiol, and prolactin response, the sum of the area under the concentration-time curve from the time of the corresponding significant rise to the end of 6 hours was calculated by using a computerized program.

Results of the GnRH-induced cycles were compared to those of hMG-induced cycles with the Student t test. Analysis of all daily results was standardized to the day of the midcycle LH peak (day 0). Correlation between daily hormone values was determined by linear-regression analysis. All values are given as mean ± SEM.

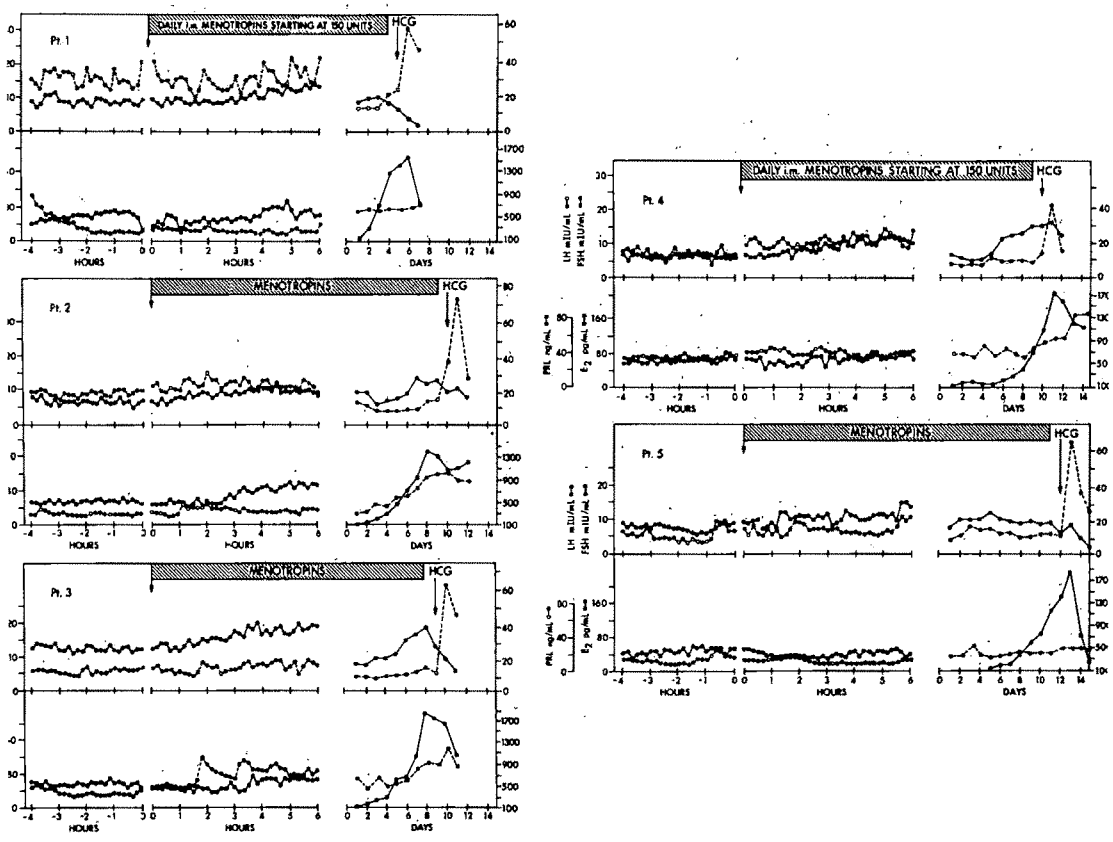


Fig. 1. Frequent (every 10 minutes) and daily concentrations of plasma LH (○) and FSH (●) *(upper panel)* and of estradiol (●) and prolactin (○) *(lower panel)* in individual patients receiving menotropins (hMG). The *scale* on the right side reflects daily levels of LH and FSH *(upper panel)* and estradiol *(lower panel)*. Time 0 hours indicates the time of initial hMG administration.

Results

Patients receiving hMG or GnRH had similar baseline levels of LH (8.3 ± 1.9 versus 6.6 ± 1.4 mIU/ml), FSH (8.3 ± 1.1 versus 6.4 ± 0.6 mIU/ml), and prolactin (19.0 ± 4.1 versus 15.0 ± 1.5 ng/ml). The plasma estradiol level was significantly lower in patients receiving hMG (54 ± 0.8 pg/ml) than in those receiving GnRH (92.6 ± 5.3 pg/ml) ($p < 0.05$). Frequent sampling before treatment revealed a variety of patterns in LH pulsatility, which would indicate that these patients represent a spectrum of abnormalities in GnRH secretion. However, both LH and FSH baselines were within the normal range for our laboratory (Figs. 1 and 2).

Approximately 150 minutes after the initiation of hMG treatment a steady and significant increase in the mean levels of plasma FSH as determined by analysis of variance was noted ($p < 0.05$). In contrast only few sporadic increases in plasma LH were found starting at approximately 3 hours after hMG administration. Furthermore, the area under the curve of plasma FSH was significantly greater than its baseline (2119 ± 240 versus 1425 ± 188 mIU/ml) ($p < 0.01$), while the increase in the area under the curve of plasma LH was of a smaller magnitude (1641 ± 283 versus 1389 ± 330 mIU/ml). Less than 2 hours after the initial and persistent FSH increase there was a significant and uniform rise in the concentration of plasma estradiol ($p < 0.05$). The area under the curve of estradiol from the rise until the end of the 6 hours (7466 ± 779 pg/ml) was significantly greater than the baseline (5613 ± 294 pg/ml) ($p < 0.05$).

The administration of pulsatile GnRH did not result in a consistent change in the levels of plasma LH, FSH, and estradiol during the initial 6 hours of treatment. However, individual patients (Numbers 3 and 4) demonstrated some increases in plasma LH, FSH, and estradiol during this time (Fig. 2). Neither stimulation regimen resulted in appreciable changes in plasma prolactin levels during the initial study period.

Mean daily levels and mean daily area under the curve of plasma LH (excluding the LH surge) in the patients receiving GnRH (18.9 ± 3.8 and 139 ± 26 mIU/ml) were not significantly different from the means found in patients receiving hMG (10.9 ± 0.9 and 91 ± 11 mIU/ml). However, mean daily levels and

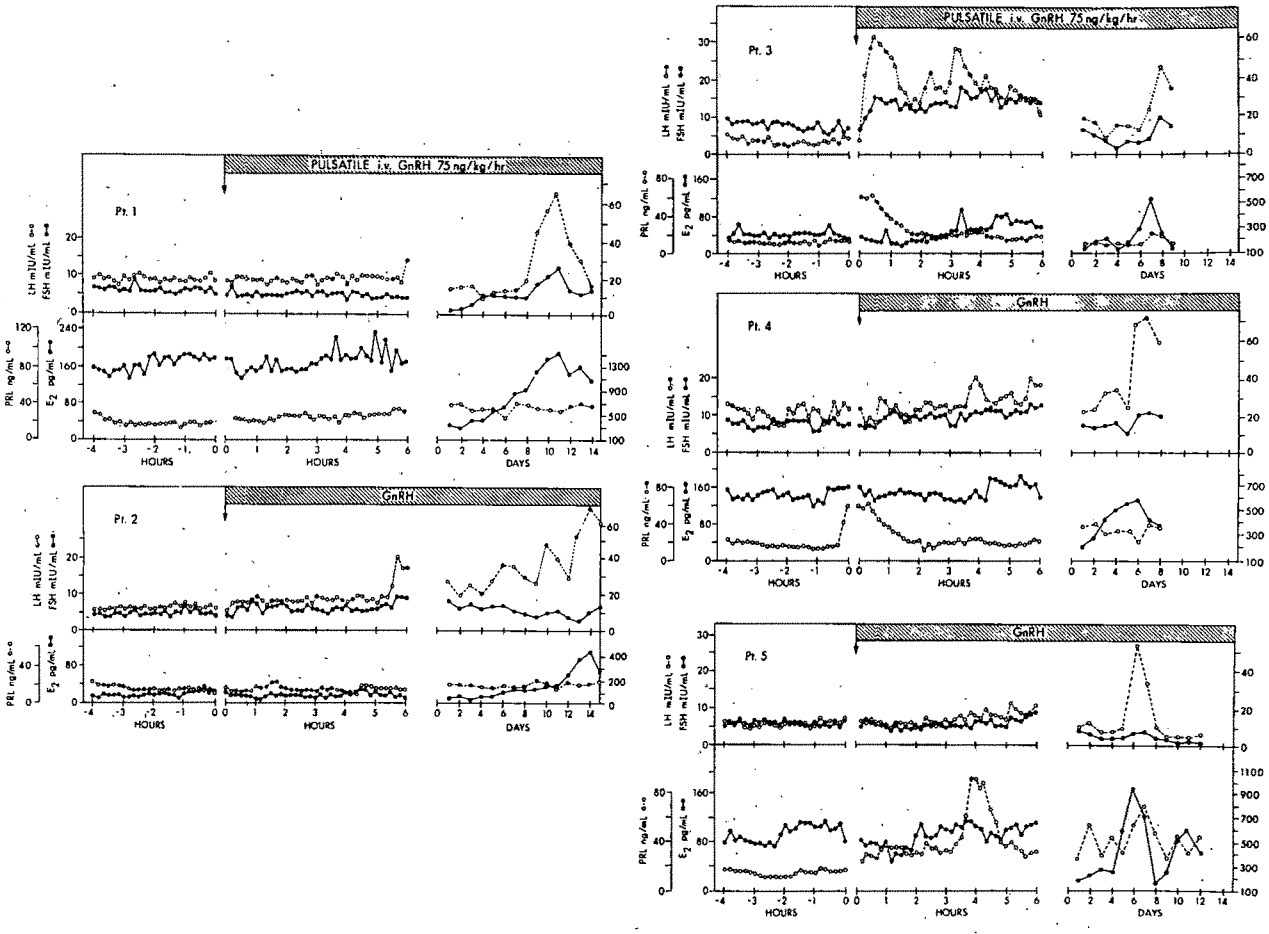


Fig. 2. Frequent (every 10 minutes) and daily concentrations of plasma LH (○) and FSH (●) *(upper panel)* and of estradiol (●) and prolactin (○) *(lower panel)* in individual patients receiving GnRH. The *scale* on the right side reflects daily levels of LH and FSH *(upper panel)* and estradiol *(lower panel)*. Time 0 hours indicates the time of initial GnRH administration.

mean daily area under the curve of plasma FSH during the same period were significantly higher in patients receiving hMG (20.0 ± 1.1 and114 ± 13 mIU/ml) than in those receiving GnRH (9.2 ± 1.4 and 53 ± 10 mIU/ml) ($p < 0.01$). Consequently, the mean LH:FSH ratio, calculated from the daily values (excluding the LH surge), was 1:2 (10.9:20.0 mIU/ml) in the hMG group and reversed to 2:1 (18.9:9.2 mIU/ml) in the GnRH-treated patients. In both groups of patients daily plasma estradiol levels increased steadily; however, during the 6 days preceding ovulation the mean plasma estradiol levels were significantly higher in the hMG group than in the GnRH-treated group (1004 ± 174 versus 495 ± 83 pg/ml) ($p < 0.05$). In addition, only in the hMG group did the mean daily plasma estradiol levels show a positive correlation with the plasma FSH levels ($r = 0.685$, $p < 0.05$) (Fig. 3).

The mean daily levels of plasma prolactin increased significantly in patients receiving hMG ($p < 0.01$). Furthermore, with the exception of Patient 1 a significant level of correlation was found between mean daily prolactin and estradiol ($r = 0.94$, $p < 0.001$) (Fig. 4). In those patients receiving GnRH a significant increase in daily plasma prolactin levels was not found, and there was no correlation between prolactin and estradiol.

The mean length of treatment required to induce ovulation did not differ between both groups (12.2 days for hMG versus 11 days for GnRH). However, those patients receiving hMG developed a mean of 4.6 mature follicles while those treated with GnRH had a mean of 1.4 follicles ($p < 0.01$). The mean largest diameter of the dominant follicle(s) for women receiving hMG was 17.7 ± 1.4 mm and for those receiving GnRH the mean was 19 ± 1.7 mm (NS).

Comment

The results of this study showed profound differences in hormonal responses to the administration of hMG and GnRH. It is conceivable that the early (<3 hours) and persistent increase in plasma FSH is the basis for hMG induction of multiple follicular stimulation that shortly thereafter resulted in a significant

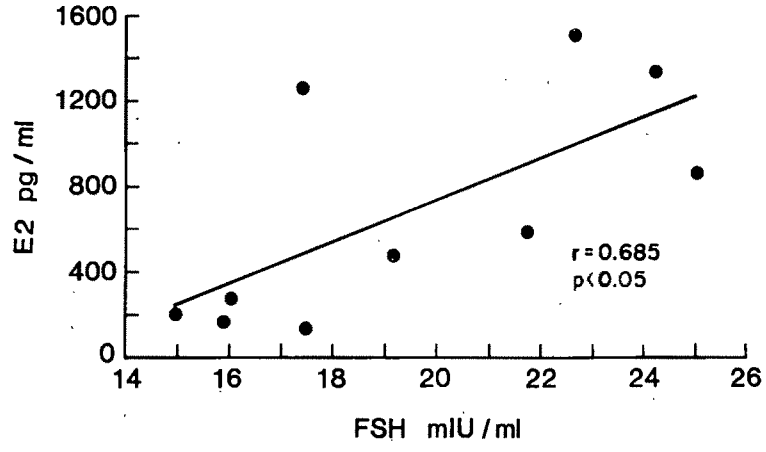


Fig. 3. Positive correlation between plasma FSH and estradiol *(E2)* in patients receiving hMG.

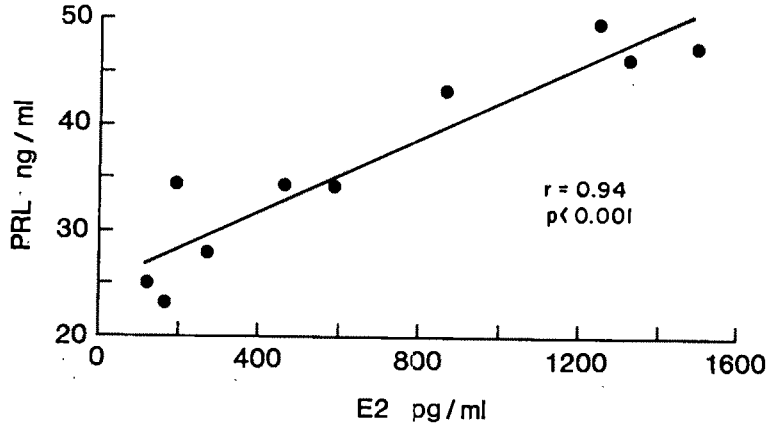


Fig. 4. Positive correlation between plasma estradiol *(E2)* and prolactin *(PRL)* in patients receiving hMG.

increase in plasma estradiol levels. This predominant FSH concentration and effect on the ovaries persisted throughout the treatment cycle as demonstrated by the reverse LH:FSH ratio that resulted in a greater number of mature follicles in the hMG-stimulated ovaries (mean of 4.6) than in the GnRH-stimulated ovaries (mean of 1.2). These results suggest that the FSH component of hMG plays a more important role than LH in initiating multiple follicular recruitment. Because of the longer half-life of FSH it is also possible that the persistent follicular stimulation by elevated FSH rescues many follicles from atresia.[15] The end result is multiple follicles that mature and ovulate if further challenged with human chorionic gonadotropin. If an excessive number of follicles is stimulated there will probably be a large amount of LH receptors. The binding of these receptors by the surrogate ovulatory dose of human chorionic gonadotropin will further stimulate those follicles, resulting in different degrees of hyperstimulation. The difference between the clearance rates of LH (30 minutes) and FSH (almost 4 hours) seems to be the cause of persistent elevated levels of FSH and only minimally elevated LH levels after the administration of a similar daily dose of each gonadotropin.[15, 16] The final blood FSH concentration and thus the degree of ovarian responsivity depends on the dose of hMG administered.

Although the LH and FSH molecular makeup of hMG is not known it seems clear that it has a significant biologic activity. It is possible that urinary gonadotropins (hMG) have a different biologic potential than those endogenously secreted after the pulsatile administration of GnRH. Although it has been reported that GnRH induces the secretion of more biologically active gonadotropins,[17] in this study a persistent ovarian response to GnRH was seen only after several days of administration. Our results do not rule out a possible role for LH in follicular development and estrogen production. Since the number of LH receptors in both human and porcine follicles has been reported to be low early in the cycle, it can be assumed that the main effect of LH is rather late in the recruitment pro-

cess.[18, 19] The progressive influence of LH is demonstrated by the absolute need for an LH signal to induce ovulation.[20] This is accomplished with human chorionic gonadotropin in hMG-treated women and with a spontaneous endogenous LH surge in GnRH-stimulated patients. Since those women with hypothalamic amenorrhea who received GnRH did not require any additional manipulation to induce ovulation, it is tempting to conclude that as long as equal pulses of GnRH are being delivered, a change in pituitary sensitivity as the result of the increasing levels of estradiol is the main pathway for the midcycle ovulatory LH surge. This is in agreement with data obtained in monkeys.[9]

It is of interest that the progressive increase in plasma estradiol levels during hMG administration was paralleled by an increase in plasma prolactin. Since these two hormones demonstrated an excellent positive correlation ($r = 0.94$, $p < 0.001$) it can be concluded that the high levels of estradiol probably directly stimulated the lactotropes. The lack of such correlation in patients receiving GnRH could be due to either the rate or the absolute degree of estradiol increase, both of which were higher in the women receiving hMG. A similar correlation has been previously reported in women treated with hMG and in pregnant women.[21, 22] However, patients receiving hMG also had higher levels of FSH and since a paracrine effect between FSH and prolactin secretion has been recently reported, such a correlation needs also to be considered.[23] However, it is doubtful that exogenously administered FSH molecules could reach and directly affect the lactotropes.

In conclusion, we have demonstrated that in women with hypothalamic amenorrhea treated with hMG but not with GnRH the concentration of FSH seems to be the main factor in the mechanism resulting in multiple ovarian follicular development and possible multiple gestation.

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Human fetal breathing response to intravenous glucose is directly related to gestational age

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This prospective longitudinal study examined human fetal breathing activity over the second half of pregnancy both in the fasting state and after intravenous glucose administration. There was a linear relationship between gestational age and percent time spent breathing after glucose between 19 and 38 weeks' gestation. However, no such correlation could be demonstrated between gestational age and fetal breathing activity in the fasting state. (AM J OBSTET GYNECOL 1987;157:1403-5.)

Key words: Fetal breathing, gestational age, glucose

Human fetal breathing movements have been recognized since 1888.[1] The development of real-time ultrasound has greatly enhanced the study of fetal activity patterns. In particular, fetal breathing movement has been used as an example of the physiology of intrauterine development, because of its possible role in preparing the fetus for extrauterine life and as a method of demonstrating fetal well-being.[2-5]

Because spontaneous fetal breathing activity is episodic in nature, with circadian and possible ultradian biological rhythms, interest has developed in stimulating fetal breathing activity by various methods, including oral and intravenous glucose administration to the mother or increasing her inspired CO_2. Previous work suggests that glucose-stimulated fetal breathing activity varies with gestational age, with a marked increase occurring some time between 24 and 36 weeks.[6] We performed the following study to delineate more sharply the development of this fetal response in clinically healthy human fetuses.

Material and methods

Clinically healthy pregnant women between 19 and 39 weeks' gestation were selected at random from a private obstetric practice and asked to participate in the study. Over a 2-year period, all pregnant nonsmokers without medical complications and with normal prepregnancy weights were included in the random selection. Subjects with an abnormal 1-hour 50 gm Glucola screen (blood glucose >135 mg/dl) were excluded. Nineteen volunteers agreed to sequential ultrasound studies during their pregnancies. The women were certain of their last normal menstrual period, and gestational age was confirmed by physical examination in the first trimester of pregnancy and/or at least one biparietal diameter ultrasound measurement. All pregnancies had progressed normally and subsequently had normal outcomes. All 1- and 5-minute Apgar scores were 8 or more. Mean birth weight was 3422 gm.

Volunteers fasted for 8 hours before the test. Subjects underwent from two to seven fetal breathing studies. Studies in the same patient were separated by at least 1 week. All studies were carried out in a dark, quiet room with the patient resting in bed in the left lateral recumbent position. Observations of the fetuses were made with a real-time ultrasound linear array scanner (Advanced Diagnostic Research, Tempe, Arizona). The hand-held transducer operated at a frequency of 3.5 mHz. Images from the scanner display screen were recorded on a videotape recorder (Sony Corporation, Japan) for analysis. After observation of the fetuses for a 20-minute control period, a bolus dose of 25 gm of 50% dextrose was administered over 20 seconds through an antecubital vein. Ultrasound observations of the fetuses were continued for an additional 60 minutes. On subsequent replay, individual fetal breathing movements were identified on the video monitor and coded on a chart recorder with an event marker as described by Patrick et al.[7] Fetal breathing activity was defined as inward movement of the chest wall echoes with outward movement of the abdominal wall echoes. Continuous breathing was considered present when the interval between breaths was less than 6 seconds. During the 80-minute observation period, fetal breathing activity as percent time spent breathing was analyzed for the four successive 20-minute periods (P_1, −20 to

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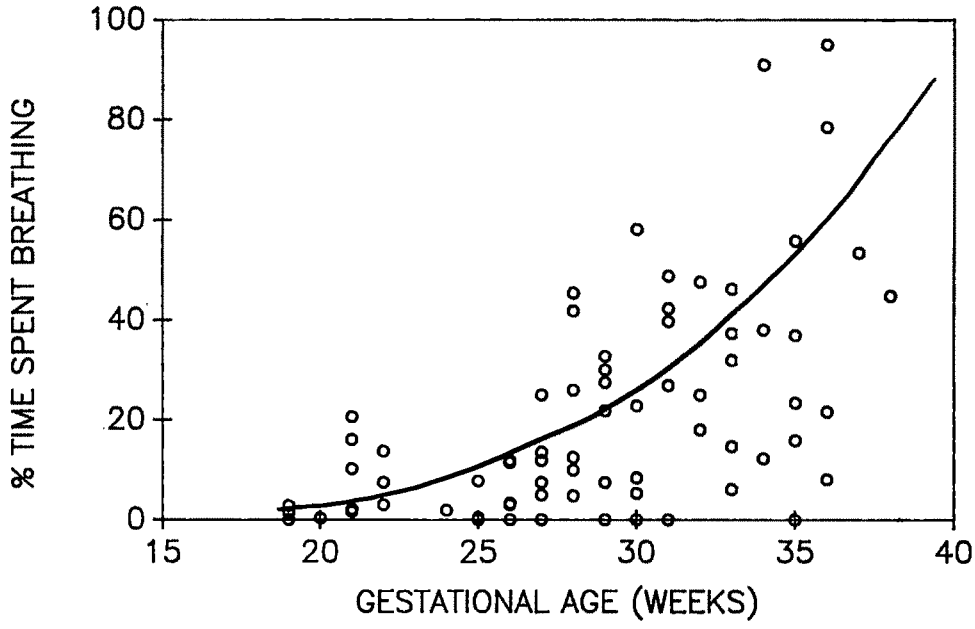


Fig. 1. Percent time spent breathing in the 40 to 60 minutes after 25 gm of intravenous glucose ($r = 0.939$; $r^2 = 0.882$).

Table I. Summary of results

Gestational age (wk)	*n*	*Percent time spent breathing (mean ± SEM)*			
		P_1 *−20-0 min*	P_2 *0-20 min*	P_3 *20-40 min*	P_4 *40-60 min*
19	3	0.5 ± 0.3	0	18.4 ± 7.4	1.4 ± 0.8
20	1	0	0	0	0.3
21	2	20.1 ± 7.0	25.0 ± 23.3	7.9 ± 6.9	8.8 ± 7.2
22	3	4.9 ± 0.5	4.6 ± 2.6	6.8 ± 2.3	8.1 ± 3.1
23	3	1.9 ± 1.0	1.9 ± 1.3	4.9 ± 1.3	11.0 ± 5.4
24	1	1.4	1.3	1.9	1.9
25	3	2.6 ± 2.3	6.9 ± 4.3	2.0 ± 1.5	2.7 ± 2.5
26	5	0.6 ± 0.2	1.2 ± 0.6	7.6 ± 6.3	5.9 ± 2.4
27	6	0.6 ± 0.4	3.3 ± 1.7	10.4 ± 5.3	12.6 ± 3.5
28	6	5.9 ± 4.4	11.8 ± 7.0	23.4 ± 4.3	23.4 ± 7.0
29	7	1.8 ± 0.7	4.1 ± 2.0	13.2 ± 5.5	17.1 ± 5.4
30	5	2.7 ± 1.2	11.0 ± 6.3	29.2 ± 13.1	18.9 ± 10.5
31	5	4.4 ± 2.6	16.0 ± 6.7	33.0 ± 9.8	31.5 ± 8.7
32	3	3.1 ± 2.0	12.6 ± 9.7	14.7 ± 13.6	30.2 ± 8.9
33	5	5.3 ± 2.4	3.5 ± 2.2	34.0 ± 5.3	27.4 ± 7.4
34	3	5.0 ± 3.0	11.7 ± 5.4	38.2 ± 18.8	43.6 ± 23.2
35	5	7.5 ± 5.5	8.0 ± 3.7	22.5 ± 3.1	26.4 ± 9.5
36	4	16.2 ± 14.6	15.0 ± 6.7	28.1 ± 11.7	50.8 ± 21.2
37	1	0	27.6	58.3	53.4
38	1	4.3	10.7	43.2	44.8

0 minutes; P_2, 0 to 20 minutes; P_3, 20 to 40 minutes; and P_4, 40 to 60 minutes). A relatively short observation period (20 minutes) may facilitate the ease of clinical application of stimulated fetal breathing studies; 20 minutes was therefore also used as the control period. Seventy-two studies were completed and all data were used in statistical analysis.

Statistical methods. A scatterplot was obtained of the raw data for each time period. Spearman rank correlation coefficients were determined for P_1 thru P_4. Regression analysis was carried out for P_4 because it had the highest correlation coefficient. Because of unequal sample sizes and unequal variance for the different gestational ages studied, regression was performed on the weighted means. Several transformations were attempted to find the formula of best fit for P_4. Multivariant analyses of variance in the SPSS and SAS programs were used to determine significance between the different time periods. A significance level of 0.05 was chosen.

Results

Spearman rank correlation coefficients showed a significant correlation between percent time spent breathing and gestational age for all three periods ($p < 0.01$) after intravenous glucose, but not in the fasting state ($p > 0.10$; Table I). The 40 to 60–minute time period had the highest correlation coefficient (0.531), so it was chosen for regression analysis to model the relationship (Fig. 1). Linear regression on the raw data yielded an r^2 value of 32.6% and an F statistic for the slope of 33.79 (1,71; $p < 0.005$). A normal probability plot of residuals was approximately a straight line. Regression analysis on the weighted means yielded the highest r^2 value (0.882) for the form: $y = -17.15 + 0.042x$ (gestational age)^2 (see Appendix).

Multivariant analysis of variance showed that 20 to 40 and 40 to 60 minutes after intravenous glucose, the percent time spent breathing by the fetus is significantly different from the percent time spent breathing in the 20-minute control period ($p = 0.001$).

Comment

This study confirms that glucose-stimulated (but not unstimulated breathing) fetal breathing activity has a significant positive correlation with gestational age in the last half of pregnancy in healthy, human fetuses. This is the first study that has attempted to model this relationship and develop regression forms that might predict fetal breathing response at various gestational ages.

Fetal breathing activity has interested investigators and clinicians for a number of reasons. The increased occurrence of this activity over the latter part of gestation is a measurable marker of maturation of a vital neonatal function. The development of this function parallels other maturational events in the fetal lung.[8] The occurrence of spontaneous breathing activity correlates with fetal well-being, and fetal asphyxia or hypoxemia is accompanied by prolonged apnea.[5] Patients in active labor are reported to have diminished fetal breathing activity, while parturients who have uterine contractions in the absence of cervical changes (false labor) have undiminished fetal breathing. Thus the presence or absence of fetal breathing has been suggested as a sign of "true labor."[9]

Because the occurrence of fetal breathing is episodic and healthy fetuses may have periods of apnea lasting 60 minutes or more, stimulation of fetal breathing, by one of several techniques, is attractive as an alternative to long spans of observation for spontaneous breathing activity. The observation of stimulated fetal breathing or its absence has been found useful in the identification of abnormal fetuses[10-13] and the prediction of the likelihood of delivery for women in true or false labor.[9]

In the evaluation of amounts of fetal breathing stimulated at various times of gestation, normative data obtained from healthy pregnancies must be developed. Our study represents an important step in the development of a longitudinal body of data for fetal breathing stimulated by an intravenous glucose infusion. Similar longitudinal studies of pregnancies complicated by hypertension or fetal growth retardation may be valuable in assessing deviations from patterns observed in health.

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Appendix

Regression form for the 40- to 60-minute period

$Y = A + B \times ^2$

Y = Percent time spent breathing 40 to 60 minutes after glucose

X = Gestational age (weeks)

A = −17.147

B = 0.043

Sonographic amniotic fluid measurement and fetal growth retardation: A reappraisal

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Sonographic estimation of amniotic fluid volume is commonly used as a diagnostic test for oligohydramnios associated with fetal malnutrition. However, methods of measurement and diagnostic criteria are controversial. We prospectively evaluated the reproducibility of amniotic fluid diameter measurement and studied various threshold definitions of oligohydramnios as predictors of small for gestational age fetuses or fetuses with low ponderal index values. An average of the vertical and two perpendicular horizontal diameters of the largest pocket of amniotic fluid was more reproducible than the maximum vertical pocket and better enabled a single observer to differentiate between patients. An average diameter of 10 mm was highly diagnostic of small for gestational age fetuses but was insufficiently sensitive (27%) to serve as a screening test. Although also of limited sensitivity (40%), an average diameter of approximately 30 mm was probably the best screening threshold. Reduced average diameter was significantly related to the occurrence of a ponderal index less than or equal to the tenth percentile for dates. Three of seven (43%) neonates with a ponderal index less than or equal to the tenth percentile and a birth weight greater than the tenth percentile for dates had a low average diameter, suggesting that reduced average amniotic fluid diameter may help to identify the malnourished fetus with birth weight greater than the tenth percentile for dates. (Am J Obstet Gynecol 1987;157:1406-10.)

Key words: Amniotic fluid, fetal growth retardation, ultrasound

Amniotic fluid volume is reduced among fetuses suffering chronic uteroplacental insufficiency with intrauterine malnutrition. In the past decade, ultrasonography has been used extensively in an attempt to estimate amniotic fluid volume and predict poor fetal growth.[1-7] However, the usefulness of this technique is controversial.[7-9] Among the various reports, the technique of amniotic fluid estimation varies from subjective to objective. Among the reports of objective assessment, technique varies from measurement of the broadest dimension of the largest pocket of fluid[2-4] to measurement of the maximal vertical depth of the largest pocket.[6,7] Criteria for "oligohydramnios" vary from <1 cm to <3 cm.[2-4,6,10] Even when objective criteria are used, some studies find amniotic fluid estimation to be a highly diagnostic test,[2] whereas others, reporting on unselected populations with a low prevalence of fetal malnutrition, have found the test insensitive and of little use as a screening tool.[3,5,7] In addition to questions of test reliability and usefulness, most of the published data on amniotic fluid measurement fail to consider the heterogeneity of aberrant fetal growth. Most reports have evaluated decreased amniotic fluid volume and the occurrence of birth weight below the tenth percentile for gestational age. Except in the postdate pregnancy,[11] the relationship of decreased amniotic fluid diameter to the occurrence of low ponderal index is largely unexplored.

These controversies prompted us to reevaluate the real-time ultrasonographic estimation of amniotic fluid volume and to reexamine its usefulness as a test for fetal malnutrition. The purposes of this study were: (1) to evaluate the reproducibility of two objective methods of measurement of amniotic fluid diameter; (2) to evaluate the relationship of the most reproducible method to the occurrence of birth weight below the tenth percentile for gestational age, as well as low ponderal index (less than or equal to the tenth percentile for dates); and to evaluate the effectiveness of various diagnostic thresholds of amniotic fluid diameter measurement as predictive tests for poor fetal growth in a population at risk.

Methods

In order to evaluate the reproducibility of amniotic fluid measurement, the frequently reported maximal vertical pocket was compared to an average of three diameters of the largest pocket of amniotic fluid. Consecutive patients were examined ultrasonographically by two independent observers after 20 weeks' gestational age. All examinations were performed with a

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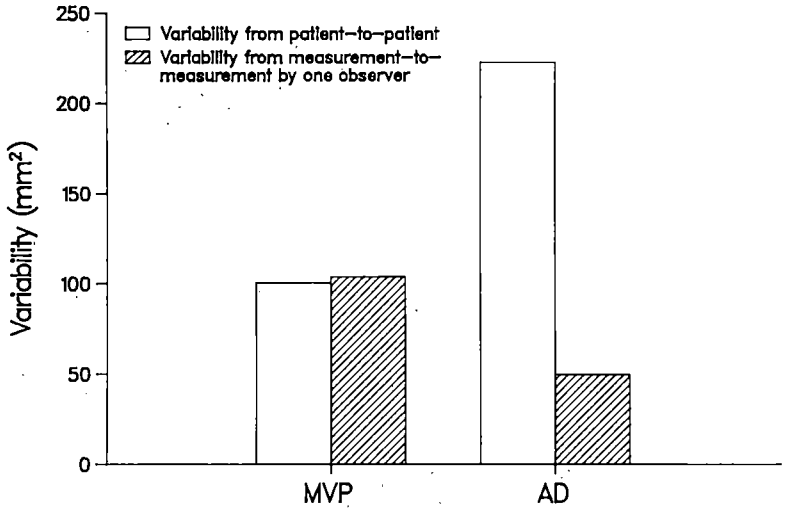


Fig. 1. Interpatient variability and intraobserver variability in the measurement of the maximal vertical pocket *(MVP)* and the average amniotic fluid diameter *(AD)*.

Table I. Sonographic measurement of the average amniotic fluid diameter and the maximal vertical pocket: variability between and within patients

	Observer 1		*Observer 2*		*Pooled*	
	MVP	*AD*	*MVP*	*AD*	*MVP*	*AD*
Mean	41.95	52.18	39.75	49.60	40.85	50.89
Coefficient of variation (%)	20.07	9.33	30.28	17.54	25.43	13.85
Variability between patients	81.40	351.10	109.10	166.77	100.57	222.76
Variability within patients	70.90	23.69	144.85	75.66	103.81	49.68

MVP = Maximum vertical pocket; AD = average amniotic fluid diameter.

General Electric RT 3000 real-time ultrasound machine equipped with a 3.5 or 5.0 MHz linear array transducer. At the beginning and at the end of each examination, all four uterine quadrants were inspected. The vertical and the two perpendicular horizontal diameters of the largest pocket of amniotic fluid that was free of umbilical cord and extremities were measured and averaged. Therefore each independent observer measured the largest pocket of fluid at the beginning and end of each examination. Neither observer was aware of which pocket was measured or of the values recorded by the other observer.

The data were evaluated by analysis of variance and by maximum likelihood estimates of the variance components between and within subjects.

Subsequently, the three diameters of the largest pocket of amniotic fluid were measured prospectively in consecutive clinic patients at risk for fetal malnutrition. Indications for examination included prepregnant weight <100 pounds, weight gain <5 pounds by 20 weeks' or <10 pounds by 30 weeks' gestational age, essential hypertension, collagen vascular disease, chronic renal disease, previous infant with birth weight less than or equal to the tenth percentile for gestational age, narcotic or tobacco addiction, or fundal height measurement lagging ≥5 weeks behind gestational age. Amniotic fluid diameter measurement did not influence patient management. In each patient, the examination done between 20 and 40 weeks' gestational age and nearest to delivery was selected for analysis.

Gestational age assignment was by best obstetric estimate based on a combination of last menstrual period, early examination, ultrasonography, and confirming neonatal Dubowitz examination. Crown-heel length measurements and Dubowitz examinations were performed at approximately 24 hours of life by trained personnel. Crown-heel length was obtained by means of the tonic-neck reflex on a measured bed. Small for gestational age or low ponderal index was defined as a birth weight or ponderal index less than or equal to the tenth percentile for gestational age.[12, 13]

After delivery, the data were evaluated by linear regression, χ^2, and Fisher's exact analyses where appropriate. Receiver-operating characteristic curve anal-

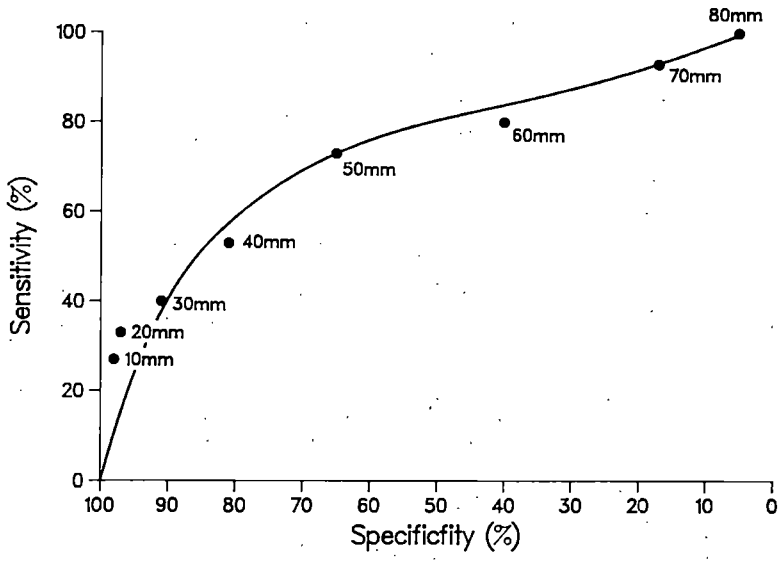


Fig. 2. Receiver-operating characteristic curve of average amniotic fluid diameter as a test for the small for gestational age fetus.

ysis was used to evaluate amniotic fluid diameter measurement as a diagnostic test for small for gestational age and low ponderal index.

Results

Initially 20 patients were examined. The means for vertical diameter and for average diameter were 40.85 and 50.89 mm, respectively. For vertical diameter, the variability between subjects was 100.57 mm^2 and the variability of the average diameter from subject to subject was 222.76 mm^2.

Within a subject, the intraobserver variability of the vertical diameter was 103.81 mm^2 with a coefficient of variation of 25.43%. In comparison, the intraobserver variability of the average diameter was 49.68 mm^2 with a coefficient of variation of 13.85% ($p = 0.2$) (Table I). There was no significant interobserver variability with either method.

Fig. 1 demonstrates that the intraobserver variability of the vertical diameter exceeded the difference between patients. Therefore a single patient with measurements done at two different times by the same observer might appear very different. In contrast, the interpatient variability of average diameter was more than four times greater than the intraobserver variability. This would enable a single observer to differentiate better between patients.

Seventy-eight patients at risk for fetal malnutrition were subsequently examined. Mean gestational age at the time of examination and at delivery was 33 ± 4 and 38 ± 3 weeks, respectively. The mean average diameter was 51 ± 21 mm. The measurement was 51.7 ± 19.2 mm among 15 patients examined before 36 weeks and 52.4 ± 21.2 mm among the 63 examined after 36 weeks. Linear correlation analysis showed average diameter to be independent of gestational age ($r = 0.1058$, $p = 0.3556$). However, it was significantly related to birth weight ($r = 0.4469$, $p = 0.0001$). Fifteen of 78 (19%) infants were small for gestational age. Crown-heel length was not measured in two neonates with early death. Eighteen of the remaining 76 (23.7%) had low ponderal index values.

The receiver-operating characteristic curve analysis evaluating average diameter as a diagnostic test for small for gestational age is presented in Fig. 2 and Table II. Although average diameter was significantly related to the occurrence of small for gestational age, it was insensitive. A threshold of 10 mm was only 27% sensitive; however, it was highly diagnostic and it represented the most efficient application of the test. At a threshold of approximately 32 mm, the sensitivity, specificity, and positive and negative predictive values were 40%, 91%, 50%, and 86%, respectively. At this cutoff point, 15% of the study population was classified as abnormal.

The relationship between an average diameter ≤32 mm and the occurrence of small for gestational age or low ponderal index value was then evaluated. The accuracy of the average diameter at predicting small for gestational age was not related to the interval between examination and delivery ($p = 0.2169$). However, the relationship between average diameter and the occurrence of low ponderal index value was significant only in 53 patients examined <3 weeks before delivery ($p = 0.0009$).

An average diameter ≤32 mm was significantly

Table II. Thresholds of average amniotic fluid diameter as diagnostic tests for the small for gestational age fetus

Average diameter (mm)	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*	*% with abnormal test (N = 78)*	*p Value*
≤40	53	81	40	88	26	0.01
≤30	40	91	50	86	15	0.009
≤20	33	97	71	86	9	0.002
≤10	27	98	80	85	6	0.004

related to the occurrence of both small for gestational age (p = 0.0341) and low ponderal index value (p = 0.0089). Average diameter was low among three of seven (43%) patients with low ponderal index values and birth weights greater than the tenth percentile and among four of six (67%) with low ponderal index values and small for gestational age (Table III).

Table III. Average amniotic fluid diameter as diagnostic test for birth weight or ponderal index less than or equal to the tenth percentile for gestational age in patients examined within 3 weeks of delivery

	Birth weight		*Ponderal index*	
	Small for gestational age	*Normal*	*Low*	*Normal*
Average amniotic fluid diameter				
≤32 mm	7	8	7	6
>32 mm	7	33	6	34
Total No. of patients	55		53	
p Value*	0.0341		0.0089	

*An average diameter ≤32 mm was significantly related to small for gestational age and low ponderal index.

Comment

Controversy exists regarding the method of amniotic fluid measurement, the definition of "abnormal amniotic fluid volume" and the type of "growth retardation" most likely to be associated with low amniotic fluid volume.[2-10] Receiver-operating characteristic curve analysis was used to evaluate diagnostic thresholds for the maximal vertical pocket in a recent report by Bottoms et al.[7] In that report, the vertical diameter was not found to be useful as a screening test for small for gestational age in a low-risk population. Interestingly, the authors postulated that the varied pathophysiologic features of atypical intrauterine growth might partially explain the variable test performance reported in the literature. For example, normal small fetuses do not have uteroplacental insufficiency, and they usually have normal amniotic fluid volume. In contrast, fetuses with malnutrition may have oligohydramnios regardless of birth weight. This controversy prompted us to evaluate the reproducibility of amniotic fluid volume estimation, the usefulness of the test in a population at risk, and the relationship between amniotic fluid status and the occurrence of poor fetal nutrition, defined as a ponderal index less than or equal to the tenth percentile or birth weight less than or equal to the tenth percentile for dates. We chose to evaluate only objective criteria for volume estimation, feeling that an objective measurement would be more widely reproducible.

Although these data do not establish the average diameter as the best of all possible measurements, they clearly demonstrate the superiority of measuring multiple diameters over measuring only one diameter. Measuring three dimensions of amniotic fluid may make the measurement more robust to changes in fetal and maternal position. Furthermore, the finding that the intraobserver variability of the vertical diameter exceeds the variability between patients provides an explanation for the poor performance of the vertical measurement reported in previous studies.

As expected, the receiver-operating characteristic curve analyses for average diameter demonstrated that the appropriate diagnostic threshold depends on the desired use for the test. The 10 mm level, which is similar to the "1 cm rule," was found to be diagnostic and efficient but was insufficiently sensitive to function as a screening test. At approximately the 30 mm level, the sensitivity, specificity, and predictive values were similar to those reported for the nonstress test, perhaps our most commonly used screening test.[14]

Because of sample truncation, we cannot fully evaluate the average diameter across all gestational ages. However, it appears to be stable at 51 ± 21 mm between approximately 28 and 40 weeks' gestational age, the time at which most examinations for growth retardation are done. This gestational age independence makes the average diameter an attractive test among patients with questionable dates.

Evaluation of the relationship between average diameter and the occurrence of ponderal index values less than or equal to the tenth percentile for dates addresses the issue of differentiating "fetal malnutrition" from "small for gestational age." Interval between the

examination and delivery significantly affected the ability of average diameter to predict low ponderal index values but had no effect on the prediction of small for gestational age. This finding is in keeping with our understanding of the continuum of intrauterine growth retardation. In a pregnancy undergoing placental senescence, a presumably well-nourished fetus first suffers depletion of fat stores. The fetus may become thin (low ponderal index value) relatively quickly and may develop low amniotic fluid volume. However, gross fetal weight declines more slowly, presumably by failure of growth. This sequence is the presumed pathogenesis of postmaturity syndrome.[10, 11] Therefore more frequent examination may be necessary to identify declining fetal fat stores. In this study, there were seven neonates with low ponderal index values and birth weight above the tenth percentile for gestational age. Forty-three percent of those had low average diameter. Thus the test identified some malnourished fetuses with apparently normal weights. These data corroborate the suggestion of Bottoms et al.[7] that the previously reported limitations of amniotic fluid measurement may be due to the varied pathophysiologic characteristics of "small for gestational age" in the populations studied. It is unreasonable to expect a normal, though small fetus to have oligohydramnios.

In summary, these data demonstrate that the average diameter of the largest pocket of amniotic fluid is more reproducible than the maximal vertical diameter and better enables a single observer to differentiate between patients. The frequently recommended threshold of amniotic fluid diameter of 1 cm was highly diagnostic and was the most efficient of the thresholds tested. However, it was insufficiently sensitive to serve as a screening test in women at high risk for fetal malnutrition. An average amniotic fluid diameter of approximately 30 mm probably represents an appropriate screening threshold for such populations. Reduced amniotic fluid diameter may help to identify the malnourished fetus who may not be small for gestational age.

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Ultrasonic prediction of birth weight in preterm fetuses: Which formula is best?

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Data from previous studies have suggested that birth weight prediction was enhanced by using formulas specifically derived from preterm fetuses. However, no prospective comparison of different formulas was performed. We obtained ultrasonic data on 61 pregnancies at risk for preterm delivery with a gestational age of 29.0 ± 3.0 weeks (mean ± SD). In all women birth weight was predicted within 7 days of delivery. Of the 61 pregnancies, 49 (80%), 41 (67%), 30 (49%), and 17 (28%) weighed <1750, 1500, 1250, and 1000 gm, respectively; 14 published formulas were compared for accuracy in predicting birth weight in these four categories. The formulas with the smallest absolute mean percent errors incorporated head and abdominal circumferences and femur length. The formula of Weiner et al., derived from low birth weight infants, produced the smallest absolute mean percent error and SD, 10.9% ± 7.9%; this error was further reduced to 7.7% ± 6.5% in infants weighing <750 gm. These findings suggest that birth weight in the preterm fetus is best predicted by a formula targeted to such a population. (Am J Obstet Gynecol 1987;157:1411-4.)

Key words: Accuracy, low birth weight, targeted formula

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Accurate sonographic estimates of fetal weight are instrumental in the management of women at risk for preterm delivery. Formulas used in predicting fetal weight have been mainly derived from measurements obtained in the term or near-term pregnancy and then extrapolated to the preterm fetus.[1-7] However, the

Table I. Ultrasonic weight-predicting formulas

Equation	*Source*	*Equation*
1	Shepard*	Log PBW = −1.7492 + 0.166(BPD) + 0.046(AC) − 0.002646 × (AC × BPD)
2	Hadlock†	Log PBW = 1.304 + 0.05281(AC) + 0.1938(FL) − 0.004(AC × FL)
3	Hadlock†	Log PBW = 1.335 − 0.0034(AC × FL) + 0.0316(BPD) + 0.0457(AC) + 0.1623(FL)
4	Hadlock†	Log PBW = 1.326 − 0.00326(AC × FL) + 0.0107(HC) + 0.0438(AC) + 0.158(FL)
5	Hadlock†	Log PBW = 1.3596 − 0.00386(AC × FL) + 0.0064(HC) + 0.00061(BPD × AC) + 0.0424(AC) + 0.174(FL)
6	Thurnau‡	PBW = (BPD × AC × 9.337) − 299
7	Weiner§	PBW = −2134.616 + 94.593(HC) + 34.227(AC)
8	Weiner§	Log PBW = 1.6575 + 0.04035(HC) + 0.01285(AC)
9	Weiner§	Log PBW = 1.6553 + 0.03986(HC) + 0.01270(AC) + 0.00265(BPD)
10	Weiner§	Log PBW = 1.6961 + 0.02253(HC) + 0.01645(AC) + 0.06439(FL)
11	Woo‖	Log PBW = 1.2758 + 0.181864(BPD) + 0.0517505(AC) − 3.34825×10^{-3}(BPD × AC)
12	Woo‖	Log PBW = 1.13705 + 0.15549(BPD) + 0.04864(AC) − 0.00279682(BPD × AC) + 0.037769(FL) − 0.000494529(AC × FL)
13	Woo‖	PBW = 15.2(BPD × AC) − 1480
14	Woo‖	PBW = 1.4(BPD × AC × FL) − 200

BPD, Biparietal diameter; AC, abdominal circumference; HC, head circumference; FL, femur length; PBW, predicted birth weight.

*Shepard MJ, Richards VA, Berkowitz RL, et al. Am J Obstet Gynecol 1982;142:47.

†Hadlock FP, Harrist RB, Carpenter RJ. Radiology 1984;150:535.

‡Thurnau GR, Tamura RK, Sabbagha RE, et al. Am J Obstet Gynecol 1983;145:557.

§Weiner CP, Sabbagha RE, Vaisrub N, et al. Obstet Gynecol 1985;65:812.

‖Woo JSK, Wan MCW, Cho KM. J Ultrasound Med 1985;4:65. Woo JSK, Wan MCW. J Ultrasound Med 1986;5:453.

Table II. Comparison of mean and absolute mean percent errors

Equation number	Source	*Error in fetuses ≤750 gm (n = 6)*		*Error in fetuses ≤1000 gm (n = 17)*		*Error in fetuses ≤1250 gm (n = 30)*	
		Mean % ± SD	*Absolute mean % ± SD*	*Mean % ± SD*	*Absolute mean % ± SD*	*Mean % ± SD*	*Absolute mean % ± SD*
10	Weiner	−4.9 ± 9.3	7.7 ± 6.5	−5.6 ± 13.4	10.4 ± 9.9	−6.0 ± 12.1	10.2 ± 8.8
4	Hadlock	1.9 ± 12.7	10.9 ± 4.6	4.3 ± 17.4	14.0 ± 10.7	5.6 ± 16.2	13.6 ± 10.1
12	Woo	0.13 ± 14.9	11.8 ± 7.4	0.56 ± 20.0	14.4 ± 13.4	2.5 ± 17.4	13.2 ± 11.3
1	Shepard	5.5 ± 15.3	13.4 ± 7.4	5.1 ± 21.8	16.7 ± 14.4	6.6 ± 18.8	15.3 ± 12.6

The absolute mean percentage difference obtained by Weiner's formula and the other three formulas is statistically significant by analysis of variance ($p < 0.05$) in birth weights <1500 gm.

Table III. Percentage of weight estimates falling within specific ranges of error in fetuses <1000 gm

Equation No.	Source	*Predicted birth weight error*		
		≤5%	*≤10%*	*≤15%*
10	Weiner	29	65	88
4	Hadlock	18	41	71
12	Woo	29	47	65
1	Shepard	18	53	59

accuracy in estimation of fetal weight has been shown to improve by the use of formulas specifically derived from a population at high risk for preterm delivery.[8-12] This enhanced accuracy may be related to the fact that "targeted" formulas account for the large head-to-abdomen ratio, a characteristic of the preterm fetus.

In the management of women at risk for preterm delivery it is important to know which formula is the most accurate predictor of weight in fetuses <2000 gm. Such information impacts on management decisions pertaining to route of delivery. Additionally, in very small fetuses size is one of the crucial factors in determining potential viability.

In this prospective study, 14 published weight-predicting formulas are compared for relative accuracy in the low and very low birth weight categories.

Material and methods

Ultrasonic fetal measurements were prospectively obtained within 1 week of delivery in 75 pregnant women who were seen consecutively and who were at high risk for preterm labor. In all patients, we used one of two ultrasound machines: an Ultramark 4 or a G.E. 3000. Echograms were obtained by one of the authors in accordance with previously described methodology, using linear array transducers with a frequency of 3.5 MHz.[13-15] The trackball located on the operating board of the ultrasonic equipment was manipulated to measure biparietal diameter, head circumference, abdominal circumference, and femur length.

Fourteen of 75 fetuses were excluded from the study because one or more of the sonographic parameters were technically unobtainable or actual birth weight was >2000 gm. Data from the remaining 61 fetuses were used to test the accuracy of 14 previously published formulas (Table I). The three most accurate formulas in the low and very low birth weight categories were identified and the birth weight estimates were compared to actual size. Additionally, comparisons were made with Shepard's formula because it was one of the first formulas used (Tables I, II, and III).

Mean percent and absolute mean percent differences between predicted and actual birth weights were calculated. The accuracy in prediction was compared in relation to 250 gm increments in size (Table II). Additionally, the percentages of infants with predicted weights falling within 5%, 10%, and 15% of actual weights were computed in the very low birth weight category (<1000 gm) (Table III).

Statistical analyses were performed with the Statistical Package for Social Sciences. Testing for significant differences in the 14 formulas was performed by analysis of variance.

Results

The mean (±SD) gestational age in the 61 fetuses studied was 29.0 ± 3 weeks. The birth weights ranged from 470 to 1965 gm with a mean (±SD) value of 1293 ± 423 gm. Of the 61 infants studied, 80% (49/61), 67% (41/61), 49% (30/61), 28% (17/61), and 10% (6/61) weighed <1750, 1500, 1250, 1000, and 750 gm, respectively.

The errors in estimation of birth weight and the coefficients of determination obtained by the three most accurate formulas and that of Shepard et al. are shown in Table II. The formula published by Weiner et al. (Equation 10, Table I) was the most accurate estimator of actual size in low birth weight infants. In contrast to results for other formulas, this accuracy was further

Error in fetuses ≤1500 gm (n = 41)		*Error in fetuses ≤1750 gm (n = 49)*		*Error in fetuses ≤2000 gm (n = 61)*		
Mean % ± SD	*Absolute mean % ± SD*	*Mean % ± SD*	*Absolute mean % ± SD*	*Mean % ± SD*	*Absolute mean % ± SD*	R^2
−8.1 ± 11.7	11.3 ± 8.5	−7.7 ± 11.2	10.9 ± 7.9	−8.6 ± 11.1	11.4 ± 8.2	0.84
4.6 ± 14.7	11.9 ± 9.7	5.4 ± 14.3	11.8 ± 9.5	4.4 ± 13.7	11.0 ± 9.2	0.83
2.0 ± 15.5	11.8 ± 10.1	3.2 ± 15.2	11.9 ± 9.9	1.8 ± 14.7	11.4 ± 9.4	0.83
5.8 ± 16.9	13.7 ± 11.3	6.9 ± 16.5	13.8 ± 11.2	5.3 ± 16.0	13.1 ± 10.5	0.81

Table IV. Example of calculations for mean percent error and absolute mean percent error

Fetus No.	*Actual weight (gm)*	*Predicted weight (gm)*	*Predicted birth weight − actual birth weight*	*Mean % error*	*Absolute mean % error*
1	1000	840	−160	−16	16
2	1000	1120	+120	+12	12
Total (n = 2)	2000	1960	−40	−4	28

Mean percent error = −4%/2 = −2%. Absolute mean percent error = 28%/2 = 14%.

improved in infants with birth weight <750 gm (Table II). Specifically, the differences in absolute mean percent errors between the formula by Weiner et al. and the other three formulas was statistically significant in weight categories <1500 gm ($p < 0.05$). The formula by Weiner et al. also had a consistently greater percentage of fetuses within a specified range of percent error (Table III); these advantages were not apparent when mean percent errors, rather than absolute mean percent errors were compared (Table II).

Comment

Different ultrasonic parameters have been used to generate a number of mathematical formulas to estimate fetal weight (Table I). However, only a few of these formulas were targeted to specific groups such as women at high risk for preterm labor or intrauterine growth retardation.[8-11] In this prospective study, we show that the formula derived by Weiner et al., which is targeted to women at high risk for preterm labor, is the most accurate method in predicting size in low birth weight fetuses (Tables I, II, and III). It should be noted that this formula was previously derived from a similar population of fetuses in the midwestern area of the country.

In previously published formulas accuracy was mainly determined by comparing the mean percent errors between predicted and actual birth weights. However, in such comparisons, negative and positive differences may cancel each other. By contrast, when the absolute mean percent error is used, the average of the sum of actual differences regardless of the directional sign is computed. In this way a true estimate of the error is obtained. The difference between mean and absolute mean percent errors in two fetuses with the same birth weight of 1000 gm is shown in Table IV; the difference is marked: −2% versus 14%.

It is apparent that the mean percent error is misleading because it reduces the difference between predicted and actual birth weights. Thus, when mean percent error is used, caution should be exercised in quantifying potential variation from actual size.

A comparison of the best three formulas in our study is shown in Table II. The absolute mean percent error, reflecting true potential difference, is consistently smaller with the formula derived by Weiner et al. Interestingly, the mean percent errors are smaller with Hadlock's formula. This is related to the fact that with Hadlock's formula the positive and negative predictions are more evenly distributed about the mean and therefore cancel each other.

Importantly, the absolute mean percent error decreases in fetuses with very low birth weight (<1000 gm) only when the formula published by Weiner et al. is used (Table II). The enhanced accuracy of the latter formula is also shown in Table III; specifically, in infants weighing <1000 gm, the predicted weight falls within 15% of the actual birth weight in 88% of cases.

Our prospective analysis also shows that birth weight is generally overestimated with the formula published by Hadlock et al. and underestimated by that of Weiner et al. (Table II). Knowledge of whether ultrasonic or even clinical estimates of birth weight tend to exceed or fall short of actual size is important.[16] For example,

in the management of a fetus near the limits of viability (25 to 26 weeks' gestation and 500 to 600 gm in weight) the decision-making process regarding possible operative intervention will partly depend on whether the method used will underestimate or overestimate birth weight.

In summary, analysis of the present series suggests that the most accurate estimates of size in low birth weight infants is obtained by using a formula targeted for that group. The formula derived by Weiner et al. is best in this respect. In evaluating the accuracy of a weight-predicting formula, emphasis should be placed on the standard deviation as well as the absolute mean percent error. Finally, knowledge of whether a particular formula is more likely to underestimate or overestimate the prediction of birth weight is helpful in particular circumstances.

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Effects of the antiprogesterone RU 486 in normal women

I. Single-dose administration in the midluteal phase

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The response to a single oral dose of the antiprogesterone RU 486 was studied in the midluteal phase in 26 normal women. Each subject received a dose between 50 and 800 mg RU 486 on days 6 to 8 after the luteinizing hormone surge and blood samples were taken over the following 48 hours. Another group of five patients received a single oral dose of 200 mg RU 486 and blood sampling was extended for 14 days. Menses were induced in all women but one within 3 days after RU 486 administration. Two distinct patient populations emerged. In nine of the subjects, there was a single bleeding episode and the treatment cycle was significantly shorter ($p < 0.05$) than the following cycle. In 16 of these 25 patients a second bleeding episode occurred 19.0 ± 0.8 days after the luteinizing hormone surge. The total treatment cycle was significantly prolonged ($p < 0.05$) when compared with the following cycle. In the group with a single bleeding episode, there was a significant decline in follicle-stimulating hormone, estradiol, and progesterone over the 48-hour sampling period, but there was no change in these values in the group with two bleeding episodes. These two groups could not be separated on the basis of RU 486 dose or serum levels. After the four higher doses, there was a dose-dependent rise in serum prolactin. There were no alterations in mean cortisol values with the three lower doses, but there was a significant increase at 24 and 48 hours after the higher doses. Serum levels of RU 486 were maximal between 1 and 4 hours and the half-life of serum RU 486 was determined to be 24 hours. (Am J Obstet Gynecol 1987;157:1415-20.)

Key words: RU 486, contraception, gonadotropins, steroids

The synthesis and development of RU 486, a 19-norprogestin derivative, is considered a major breakthrough in steroid endocrinology[1] and has opened a new era in fertility control. Beyond its potential use in contraceptive technology, the antiprogestational[1] and antiglucocorticoid[2-4] activities of RU 486 serve as a unique tool for investigation of hormone action.

In early reports, RU 486 has been noted to interrupt the luteal phase in women[4-6] and in monkeys[6] and to terminate early pregnancy.[7, 8] RU 486 has also been shown to have an affinity for the glucocorticoid receptor that is about four times higher than that of dexamethasone and has been shown to have antiglucocorticoid activity. After a single oral dose of RU 486, there is a transient increase in adrenocorticotropic hormone.[2, 3]

The aim of this study was to investigate the effect of a single oral dose of RU 486 on bleeding, gonadotropin, and steroid patterns given to healthy women in the midluteal phase. In addition, the pharmacodynamics of this compound were studied with a recently developed radioimmunoassay.

Material and methods

Thirty-one healthy women, whose ages ranged from 22 to 35 years, were selected for participation in this study. They had regular menstrual cycles (28 ± 3 days) and were within 15% of their ideal body weight. They either had been surgically sterilized or had used a barrier method for contraception. The volunteers had not taken any steroid medication within the last 6 months.

On the tenth day of the menstrual cycle, each patient began to undergo daily blood sampling for determination of the day of the luteinizing hormone (LH) surge. Six to eight days after the LH surge, the volunteers were asked to report at 7:30 AM, after an overnight fast, and to refrain from smoking after midnight for administration of medication.

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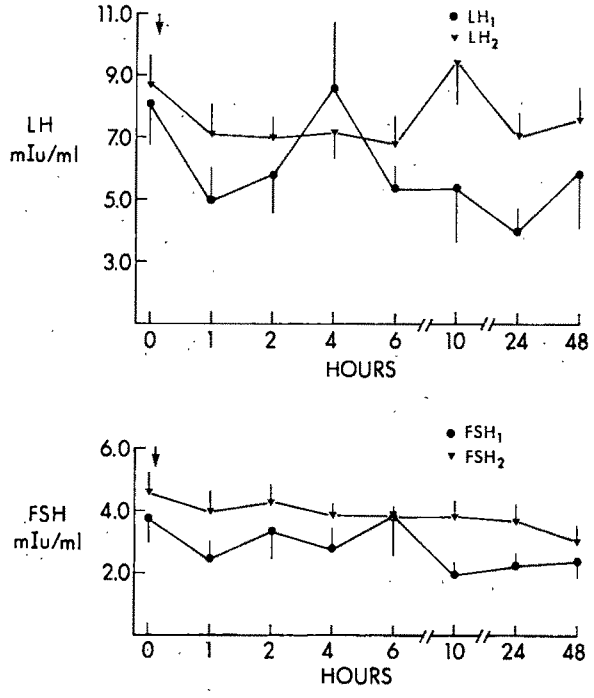


Fig. 1. LH *(upper panel)* and FSH *(lower panel)* values after a single oral dose of RU 486 ranging from 50 to 800 mg. LH_1 and FSH_1 refer to the patients with one bleeding episode after RU 486 administration and LH_2 and FSH_2 refer to patients having two bleeding episodes. No significant changes in LH were noted in either group. Analysis of variance detected a significant decrease in FSH_1 ($p < 0.001$) over the 48-hour sampling period.

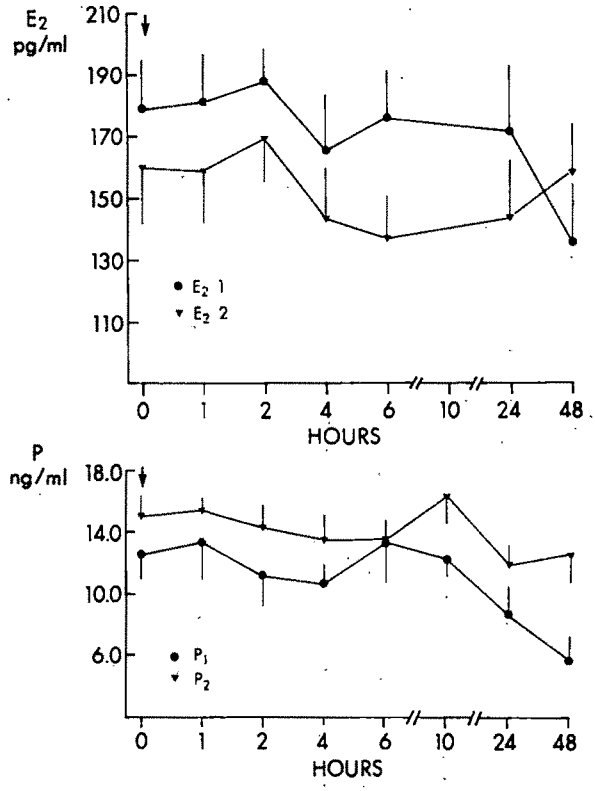


Fig. 2. *Upper panel*: Estradiol (E_2) values after a single oral dose of RU 486 ranging from 50 to 800 mg. $E_2 1$ refers to patients with one bleeding episode after RU 486 administration and $E_2 2$ refers to those with two bleeding episodes. Analysis of variance detected a significant decrease in $E_2 1$ ($p < 0.05$) over the 48-hour sampling period. *Lower panel*: Progesterone *(P)* values after a single oral dose of RU 486 ranging from 50 to 800 mg. P_1 refers to patients with one bleeding episode after RU 486 administration and P_2 refers to those with two bleeding episodes. Analysis of variance detected a significant decrease in P_1 ($p < 0.005$) over the 48-hour sampling period.

Subjects received either 50, 100, 200, 400, 600, or 800 mg of RU 486 (50 mg tablets supplied by Roussel-Uclaf, Romainville, France) at 8:00 AM. All groups comprised four subjects except for the two highest-dose schedules where five subjects were studied in each group. No smoking or food was permitted for 4 hours after ingestion of the medication and vital signs were recorded every 30 minutes for 6 hours. Blood samples were obtained through an indwelling catheter at −½, 0 +½, 1, 2, 4, 6, 10, 24, and 48 hours. A further group of five subjects received 200 mg as a single oral dose and blood sampling was continued daily for 1 week and then was performed at 10 and 14 days (extended sampling group).

These blood samples were assayed for prolactin, estradiol, progesterone, cortisol, LH, follicle-stimulating hormone (FSH), and RU 486 by previously described radioimmunoassay methods.[9-12] Normal serum AM cortisol values are 10 to 30 μg/dl. Two different radioimmunoassays were used to measure RU 486. The first was the method of Salmon and Mouren.[11] The second utilizes chromosorb column chromatography.[12] This latter, more specific assay was used only to measure RU 486 levels in the samples from the extended sampling group. The antiserum against RU 486 used in both assays was donated by Roussel-Uclaf. In addition, SMA-18 and complete blood count were performed at 0, 4, 10, 24, and 48 hours.

Results were analyzed by analysis of variance with the use of BMDP Statistical Software.[13] One- and two-way analysis of variance and Student's *t* test were used to determine statistical differences between groups.

Results

Clinical features. Menses commenced in all patients but one within 1 to 3 days after RU 486 administration (Table I). In nine of these 25 subjects (two who received 50 mg, two who received 100 mg, one who received 200 mg, one who received 400 mg, and three in the 800 mg group), this represented the only bleeding episode. The mean length of the treatment cycle in these nine subjects was significantly less ($p < 0.05$) than that of the following cycle (Table I).

In the remaining 16 subjects, a second bleeding episode was reported. In these subjects the initial bleeding episode occurred at a similar time after the LH surge as in the previous group, but the duration of bleeding was significantly shorter ($p < 0.05$). The full duration of the treatment cycle in those subjects who had two bleeding episodes (with the onset of the second bleeding episode used as a reference) was 32.9 ± 1.1 days. This was longer ($p < 0.05$) than the following cycle (Table I).

In none of these subjects were any adverse effects encountered. Furthermore, electrolytes, hepatic function, and blood count did not change in any subject. In one subject who received the 600 mg dose, bleeding did not occur until 56 days after treatment.

Hormonal parameters. The hormonal profiles of four patients (two from each group) were excluded from analysis because of incomplete data. In those 14 subjects who had two bleeding episodes, there was no change in serum LH, FSH, estradiol, or progesterone over the 48-hour sampling period. However, in those seven subjects who had a single bleeding episode, analysis of variance showed a significant decline in estradiol ($p < 0.05$), FSH ($p < 0.001$), and progesterone ($p < 0.005$) (Figs. 1 and 2).

There were no significant changes in LH, FSH, estradiol, or progesterone when hormonal results were expressed according to the dosage schedule of RU 486 and independent of the bleeding pattern. Analysis of variance showed that there was a significant increase in prolactin ($p < 0.05$) with the 50, 200, 400, and 800 mg dosages. When compared with the pretreatment levels, the peak values were greatest between 4 and 6 hours ($p < 0.05$). Values returned to normal by 24 hours (Fig. 3).

Analysis of variance showed a significant increase ($p < 0.05$) in mean cortisol values after the 100, 400, 600, and 800 mg doses. When compared with baseline values, the cortisol level rose significantly ($p < 0.05$) at 24 hours after the 600 and 800 mg doses and at 48 hours with all three higher doses (Fig. 4).

RU 486 levels. When measured directly after diethylether extraction, the serum levels of RU 486 reached a maximum between 1 and 4 hours after ingestion of all doses (Fig. 5). All RU 486 levels were significantly lower with the 50 mg dose than with the five higher-dose regimens ($p < 0.05$). The RU 486 levels at 6, 24, and 48 hours were also significantly lower after the 100 mg dose than with the four higher doses ($p < 0.05$). RU 486 levels were the same with doses of 200 to 800 mg. With doses of ≥200 mg, mean serum RU 486 levels fell minimally, and even after 48 hours values were still above 1.4 μg/ml. On the other hand, after the 50 and 100 mg doses, RU 486 values did decrease and were under 0.4 μg/ml at 48 hours. There were no differences in the RU 486 levels between the groups with one or two bleeding episodes.

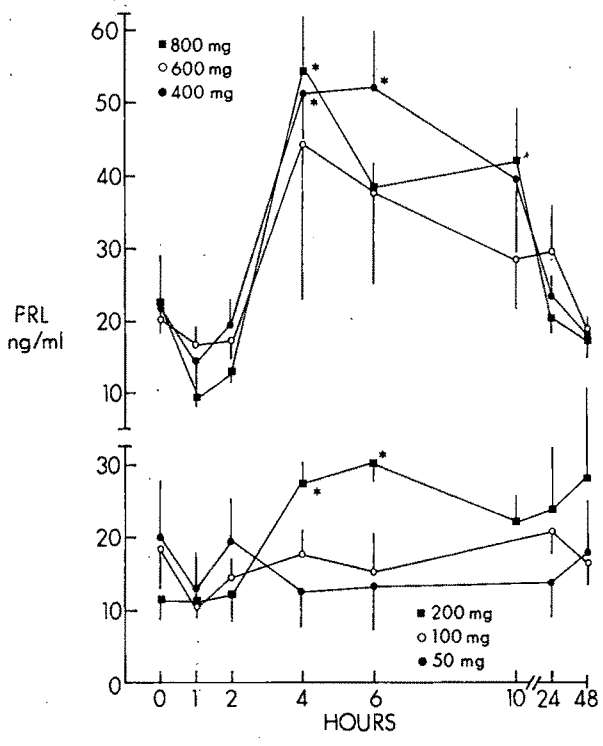


Fig. 3. Prolactin *(PRL)* levels after a single oral dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected a significant increase in *PRL* after the 50, 200, 400, and 800 mg doses, * $p < 0.05$ increase over baseline values as determined by the paired *t* test.

The serum levels of RU 486 in the extended sampling group are shown in Figs. 5 to 6. Chromosorb column chromatography was used in these samples and the values were lower than those with corresponding 200 mg dosage schedule. Nevertheless, after 4 hours the disappearance curves with both assays were parallel. Presumably, the values after chromatography represent true RU 486 concentrations. The half-disappearance time was 24 hours (Fig. 6).

Comment

Administration of RU 486 as a single oral dose from 50 to 800 mg in the early luteal phase was well tolerated. The induction of menses within 3 days is in accordance with results of previous studies.[5,6,15] This vaginal bleeding, which occurred despite high levels of estradiol and progesterone, implies that RU 486 acts directly on the endometrium to produce shedding regardless of any effect it may have on hormonal levels. A similar bleeding pattern has also been documented in nonpregnant women during concomitant treatment with RU 486 and human chorionic gonadotropin in the luteal phase.[6-14]

A total of 33% of our subjects displayed only a single

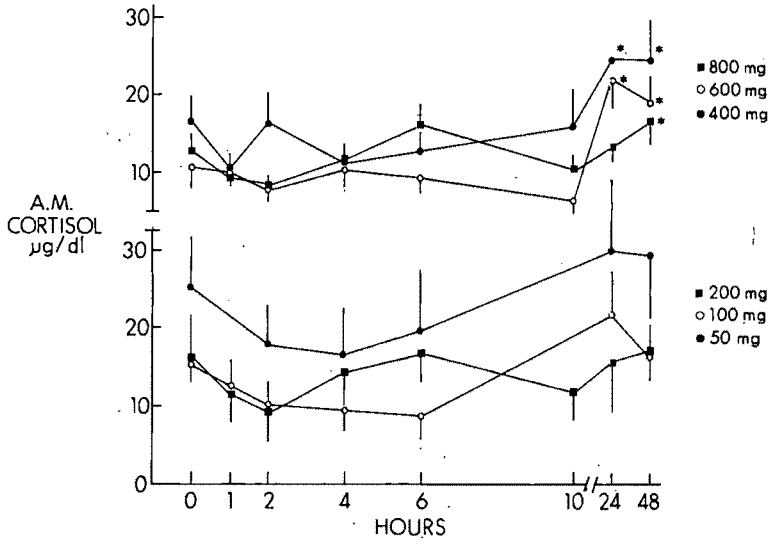


Fig. 4. Cortisol values after a single dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected a significant increase in cortisol ($p < 0.05$) after the 100, 400, 600, and 800 mg dose. * $p < 0.05$ increase over baseline values as determined by the paired t test.

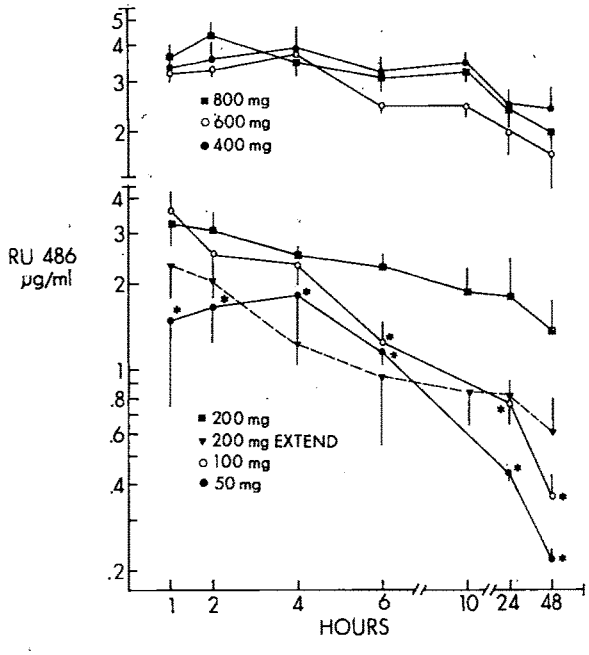


Fig. 5. RU 486 levels after a single oral dose of RU 486 ranging from 50 to 800 mg. Analysis of variance detected that all points after 50 mg dose and at 6, 24, and 48 hours after the 100 mg dose were significantly lower ($p < 0.05$) than values after the higher doses. The *200 mg extend* refers to the extended sampling group that is also depicted in Fig. 6.

bleeding episode, whereas in the remaining subjects a second bleeding episode occurred. This phenomenon appeared to be independent of the dose or serum concentration of RU 486. The hormonal profile in these two groups was distinctly different. In those subjects with two bleeding episodes there were no changes in LH, estradiol, or progesterone levels. In addition, if the commencement of the second bleeding episode is regarded as the termination of the cycle, the luteal phase appeared to be prolonged by RU 486. In contrast, in those subjects who had a single bleeding episode there was a progressive significant decline in serum FSH, estradiol, and progesterone levels over 48 hours of sampling time with a concomitant shortening of the luteal phase and cycle length. This suggests that under certain circumstances RU 486 can be luteolytic. Similar observations have been made by other workers.[6, 15] Although it is possible that the decline in steroid levels may represent the fall seen in the latter part of the normal menstrual cycle, this is unlikely since no corresponding fall occurred in the group with two bleeding episodes.

The decrease in the serum progesterone level could be related to a direct action of RU 486 on ovarian steroidogenesis.[17] However, it could also represent an effect on the hypothalamic-pituitary axis. We did observe a decrease in FSH in one group of patients but were unable to detect a decrease in LH secretion. Recently, with more frequent sampling we also observed a reduction in amplitude and frequency of LH pulses during RU 486 administration in the luteal phase.[18] Other workers have also described a reduction in LH pulse amplitude consequent to RU 486.[15]

Nieman et al.[6] observed menstrual bleeding in normal women within 72 hours of a single oral dose of RU 486 (10 mg/kg body weight) given in the midluteal phase. In the patients treated with RU 486 on luteal day 7, they report a decrease in the serum progesterone level by luteal phase day 10 and the onset of vaginal

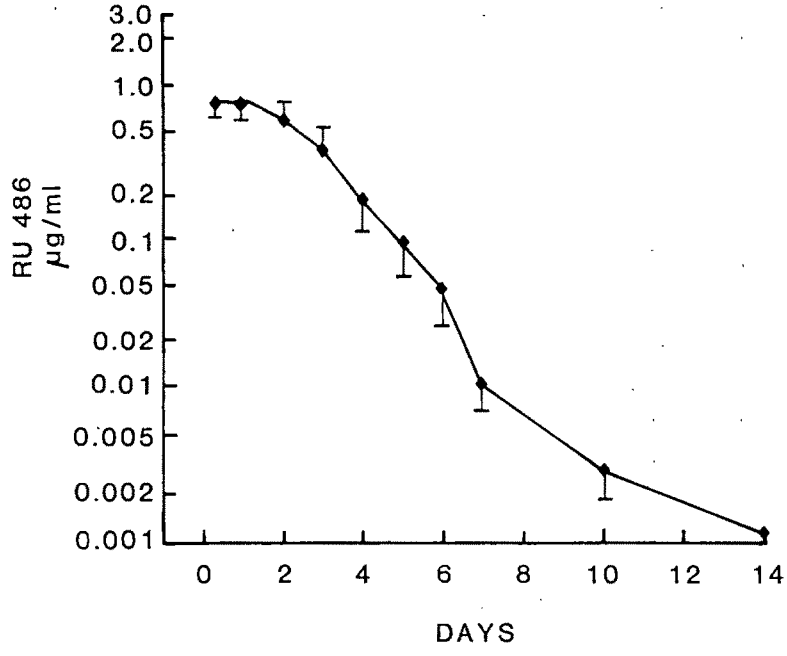


Fig. 6. RU 486 levels in the extended sampling group after a single oral dose of 200 mg RU 486. The half-life of RU 486 is 24 hours.

Table I. Effects of RU 486 administration

Bleeding episodes	*N*	*Onset (days after LH surge)*	*Days of bleeding*	*Treatment cycle length (days)*	*Posttreatment cycle length (days)*
1	9	9.8 ± 0.6	3.4 ± 0.3	25.2 ± 1.4*	34.0 ± 2.1
2	16	9.6 ± 0.5	6.0 ± 0.8†	32.9 ± 1.1	26.6 ± 1.7
		19.0 ± 0.8	4.2 ± 0.3		

Values are mean ± SD.
*$p < 0.05$ when compared with posttreatment cycle length.
†$p < 0.05$ when compared with group with one bleeding episode.

bleeding within 72 hours of treatment in all patients. They also described two patient groups as regards to the onset of menses, which may correlate with our two patient groups.

Circulating RU 486 levels measured by immunoassay showed that this synthetic steroid was absorbed rapidly. Doses of 50 and 100 mg gave lower blood levels than higher doses. However, no clear dose response was evident since 200 to 800 mg RU 486 gave similar blood levels. The immunoassay used to determine these circulating levels is nonspecific and measures metabolites, including the monodemethylated and didemethylated compounds as well as the alcoholic derivative.[11, 12] These metabolites seem to have a smaller biologic activity.[19] For this reason we developed a more specific radioimmunoassay using preliminary chromosorb chromatography. With this modification, values were lower but gave a parallel disappearance curve. Values of RU 486 measured by immunoassay after chromosorb chromatography give values similar to those determined by high-pressure liquid chromatography.[12] The half-life of RU 486 was 24 hours. The long half-disappearance of RU 486 suggests that there is binding to plasma proteins. Recent studies have shown that RU 486 binds to an α_1-globulin.[19]

The late rise in cortisol, noted at 24 and 48 hours after ingestion of the 400, 600, and 800 mg doses, suggests the presence of a mild degree of antiglucocorticoid activity with these doses. Nevertheless, mean values remained within the normal range. The late increase seen after 24 and 48 hours, without any change during the initial 10 daytime hours after ingestion of the drug, would be consistent with the work of Gaillard et al.,[3] who demonstrated that the antiglucocorticoid activity is seen only during the early morning hours when adrenocorticotropic hormone secretion is high.

Rakoff and Yen[16] reported a transient increase in prolactin in estrogen-primed women receiving in-

tramuscular progesterone after oophorectomy. This would imply a positive effect of progesterone on prolactin secretion, which is in contrast to the transient increase in prolactin we noted after treatment with an antiprogesterone. Healy et al.[20] described a decrease in prolactin after treatment with RU 486 in monkeys (10 mg/kg).[20] However, these were castrated monkeys who had steroid replacement via estradiol and progesterone capsules, which produce basal hyperprolactinemia. Within 1 hour after parenteral RU 486 administration, these elevated prolactin levels were decreased. Thus the effect of RU 486 on prolactin secretion varies and may depend on the mode of its administration, the dose, the species, and the prevailing steroid concentrations.

The reason why two subgroups emerged in this study is not evident. They could be differentiated by both bleeding patterns and hormonal levels. Both groups received RU 486 on days 6 to 8 after the LH surge and there were no differences in RU 486 serum levels between the two groups. Thus these two patterns of response could not be differentiated on the basis of time of treatment, dosage of RU 486, or circulating RU 486 levels.

RU 486 has been proposed as a once-a-month pill.[6] Our data would support the value of RU 486 for this use. However, the emergence of two patient groups suggests that further characterization of these distinctive patterns is necessary.

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Effects of the antiprogesterone RU 486 in normal women

II. Administration in the late follicular phase

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RU 486, a synthetic steroid with antiprogesterone receptor activity, was used to investigate the importance of progesterone on gonadotropin secretory dynamics in the midcycle of the normal menstrual cycle. Six normally cycling women were followed for three consecutive cycles. During each cycle, blood samples were obtained beginning on day 10 and continued until menses. After a control cycle, 100 mg RU 486 was given orally between days 10 and 17. The patients were followed for a posttreatment cycle with no medication. When RU 486 was given before the midcycle, the luteinizing hormone surge was delayed by 15.0 ± 2.1 days after ingestion of the last pill, resulting in cycles of 40.6 ± 2.6 compared with 28.0 ± 2.3 days ($p < 0.01$). During RU 486 administration and at the time a normal luteinizing hormone surge was anticipated, an attenuated luteinizing hormone/follicle-stimulating hormone surge was noted that was not followed by a rise in progesterone. After the attenuated surge a normal luteinizing hormone/follicle-stimulating hormone level occurred, with a normal rise in progesterone. Estradiol levels during RU 486 administration decreased during treatment, indicating a possible direct action of RU 486 on the ovary. (Am J Obstet Gynecol 1987;157:1421-6.)

Key words: RU 486, menstruation, gonadotropin

The precise mechanisms that underlie the processes of ovulation are not known, but two factors play important roles. The rapid rise of estrogen through a positive feedback action on the hypothalamic-pituitary unit triggers the midcycle surge. Also, the small increase in preovulatory progesterone levels[1] appears to amplify the pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) at midcycle.[2-4] RU 486, a synthetic steroid with antiprogesterone receptor activity,[5] was used to assess the importance of progesterone on midcycle secretory dynamics.

Material and methods

Six women ages 21 to 34 years with cycle length 28 ± 2 days were followed for 3 consecutive cycles. During each cycle, blood was drawn starting on day 10 and continued daily until the next menses. Bleeding records were kept during the study period. After the pretreatment cycle, 50 mg of RU 486 was administered twice a day (50 mg tablets supplied by Roussel-Uclaf, Romainville, France) from day 10 through 17. The selection of this dose was based on a dose-response study.[6] During the posttreatment cycle the patient received no medication, but blood sampling continued as in the two previous cycles.

Blood samples were allowed to clot and sera were separated and stored at −20° C until assayed for LH, FSH, estradiol (E_2), and progesterone by previously reported radioimmunoassay.[7-9] Computer assistance by Clinfo program and BMDP Statistical Software[10] was used for statistical analysis with area under the curve, the Kruskal-Wallis nonparametric test, the paired t test, and one- and two-way analysis of variance when appropriate. The area under the curve was determined by the trapezoidal rule and included values on days −2 through +2 after the LH surge for determination of LH and FSH, days −2 through 0 for E_2, and days 0 through +14 for progesterone.

Results

Administration of RU 486 during the late follicular phase delayed development of a normal LH surge an average of 15.0 ± 2.1 days from the time of the last tablet (range 10 to 21 days). This resulted in a follicular phase of 28.0 ± 2.3 days, which was significantly longer than the follicular phase in the control cycle of 14.6 ± 1.3 days ($p < 0.005$). During the treatment

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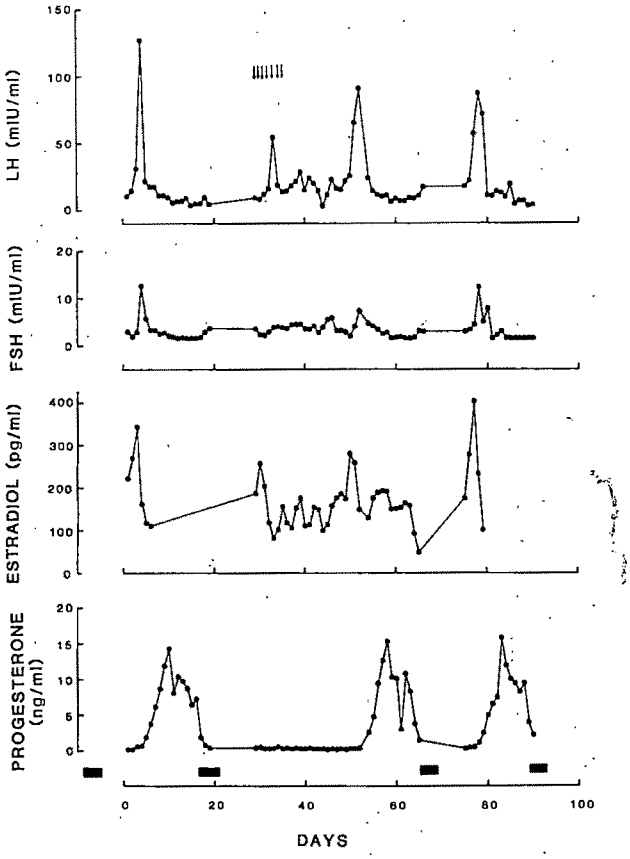


Fig. 1. LH, FSH, and progesterone values over the 3-month study period in a typical patient. *Arrows* indicate treatment days and *solid bars* indicate bleeding days. An attenuated surge occurs during the time of RU 486 administration and is followed by a normal LH surge.

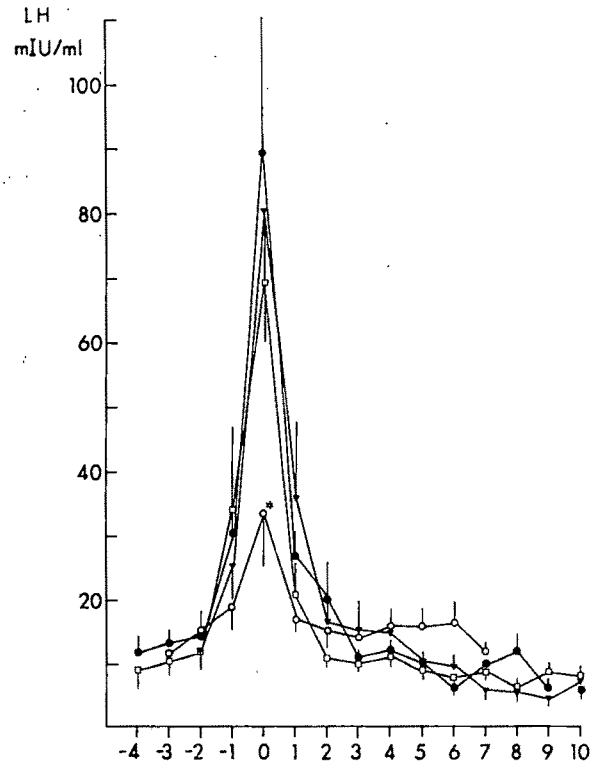


Fig. 2. LH values normalized to the day of the LH surge during the pretreatment month (▼) the attenuated (○) and normal (●) surges after RU 486 treatment, and the posttreatment cycle (□). The values of the attenuated surge are significantly lower on day 0 than before treatment ($p < 0.05$).

month, the interval between the normal LH surge and the occurrence of menses was 13.8 ± 1.2 days and not different from the pretreatment luteal phase length. The total treatment cycle length after RU 486 administration averaged 40.6 ± 2.6 days, compared with 28.0 ± 2.3 days during the pretreatment cycle ($p < 0.01$; Table I).

The bleeding pattern and serum LH, FSH, progesterone, and E_2 values of a representative subject are shown in Fig. 1. In the pretreatment cycle shortly after blood sampling began, the normal LH surge was followed by a rise in serum progesterone levels compatible with a normal luteal phase pattern. The length of this subject's pretreatment cycle was 29 days. During the following cycle RU 486 was administered during days 10 through 17. On day 14 into the treatment cycle, and during the time an LH surge was expected, an attenuated LH surge was noted that was smaller than the normal LH surge of the pretreatment cycle and was not followed by any rise in progesterone levels.

Nineteen days after the attenuated surge, a normal LH surge was noted that was followed by a rise in serum progesterone compatible with a normal luteal phase. There was no vaginal bleeding between the time of the attenuated surge and the treatment normal surge. The next bleeding episode occurred after the fall in serum progesterone, and this resulted in a total cycle length for the treatment cycle of 49 days. During the posttreatment cycle, a normal LH surge occurred on day 13 and the total cycle length was 26 days. All six patients demonstrated similar patterns.

Fig. 2 shows the mean (±SEM) LH values for all six patients normalized to the day of the LH surge (day 0). The LH surge of the pretreatment cycle, the treatment normal surge, and the surge during the posttreatment cycle are similar in magnitude. However, the attenuated surge occurring during RU 486 administration was significantly less. Kruskal-Wallis one-way analysis of variance detected LH values on day 0 to be significantly lower than control values ($p < 0.05$). These data were also confirmed by calculation of areas under the LH curve (Fig. 3).

Similar data for FSH are shown in Figs. 3 and 4. Values of FSH on days 0 and +1 were significantly smaller ($p < 0.001$) during the attenuated surge com-

pared with pretreatment values as determined by one- and two-way analyses of variance. Calculation of the areas under the FSH curves also confirmed these findings (Fig. 3).

E_2 values normalized to the day of the LH surge are shown in Fig. 5. When taken together, values on days -1 and 0 were significantly less ($p < 0.005$) than pretreatment values. Areas under the curve for E_2 during the attenuated surge were significantly less than the pretreatment, normal treatment, and posttreatment surge values.

Fig. 6 shows the progesterone values normalized to the day of the LH surge. Progesterone values preceding the attenuated surge were significantly lower ($p < 0.01$) on days -1 and 0 compared with pretreatment and posttreatment values when analyzed by analysis of variance. Progesterone levels from days 1 through 8 after the attenuated LH surge were also significantly lower ($p < 0.01$) than pretreatment values. However, once a normal LH surge occurred after RU 486, it was followed by a normal rise in progesterone during the next 14 days. The total area under the progesterone curve after the treatment normal surge was not different from the values after the pretreatment or posttreatment surges, whereas the area under the curve after the attenuated surge was significantly less ($p < 0.01$).

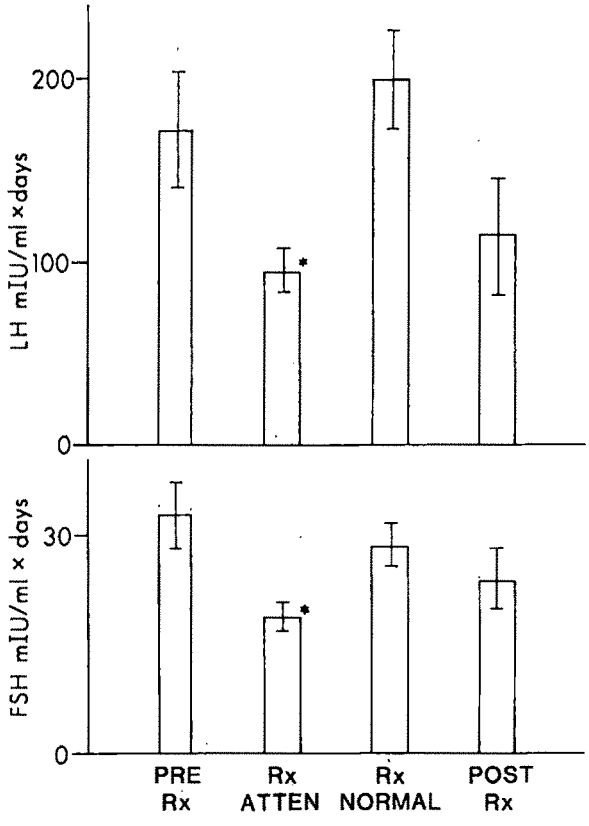


Fig. 3. Areas under the LH and FSH curve in the pretreatment month *(PRE-Rx)*, during the attenuated LH surge *(Rx ATTEN)*, in the normal LH surge occurring during the RU 486 treatment cycle *(Rx NORMAL)*, and in the posttreatment month *(POST Rx)*. $*p < 0.05$.

Comment

Administration of RU 486 during days 10 through 17 of a normal menstrual cycle prevented the development of a normal LH/FSH surge and delayed formation of a new surge by an average of 15 days. This confirms the ability of RU 486 to effectively block the midcycle gonadotropin surge, delay follicular development, and prolong menstrual cycle length.[11-14] This effect is most probably related to its antiprogesterone activity and not its antiglucocorticoid activity, as this effect is not altered by concomitant administration of dexamethasone in primates.[13] Our data support the possibility that progesterone antagonism affects the hypothalamic-pituitary axis by inhibiting the normal positive effect of progesterone on gonadotropin secretory dynamics at midcycle. RU 486 has been reported to have a mild progestational effect, and similar to the suppressive effect of progestins in oral contraceptives, the effect of RU 486 on midcycle gonadotropin may be due to progesterone agonism.[13] However, the dominant effect of RU 486 is that of an antiprogesterone, and it is more likely that suppression of the LH surge is due to progesterone antagonism.

In addition, there was a significant reduction in E_2 and progesterone levels before the day of the attenuated LH/FSH surge. These data suggest that RU 486 may have a direct inhibitory effect on ovarian steroidogenesis. In vitro studies support a direct action of RU 486 on ovarian E_2 and progesterone production.[15, 16] However, it may also be the case that lower FSH levels inadequately stimulate ovarian E_2 production. A decline in FSH levels after RU 486 was noted in this study and in other studies.[6]

The 15-day delay from the time of the last tablet to the onset of a normal LH surge suggests that recruitment of another dominant follicle is required to initiate ovulation after what appears to be ablation of the dominant follicle by RU 486 administration. This is supported by recent ultrasonographic studies of follicular development after RU 486.[14] However, it is plausible that the dominant follicle may not be ablated as such and may merely be arrested temporarily and undergo spontaneous recovery after RU 486 administration ceases. Further ultrasonographic studies may better characterize follicular development.

Successful blockage of the LH/FSH surge has been reported in humans[11] and in monkeys[12, 13] when treated in the late follicular phase. These data confirm the abil-

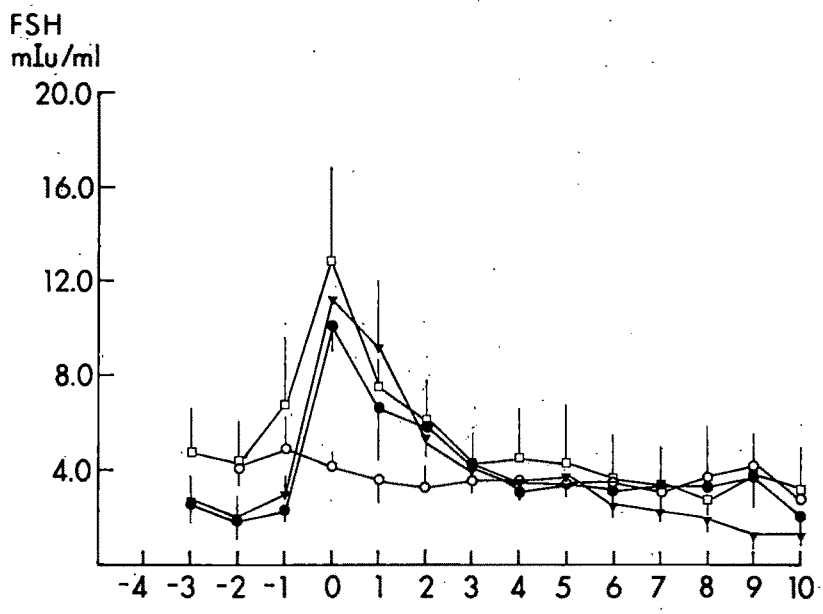


Fig. 4. FSH values normalized to the day of the LH surge during the pretreatment month (▼) the attenuated (○) and normal (●) surges after RU 486 treatment, and the posttreatment cycle (□). FSH values on days 0 and +1 were significantly less ($p < 0.001$) during RU 486 treatment than before treatment.

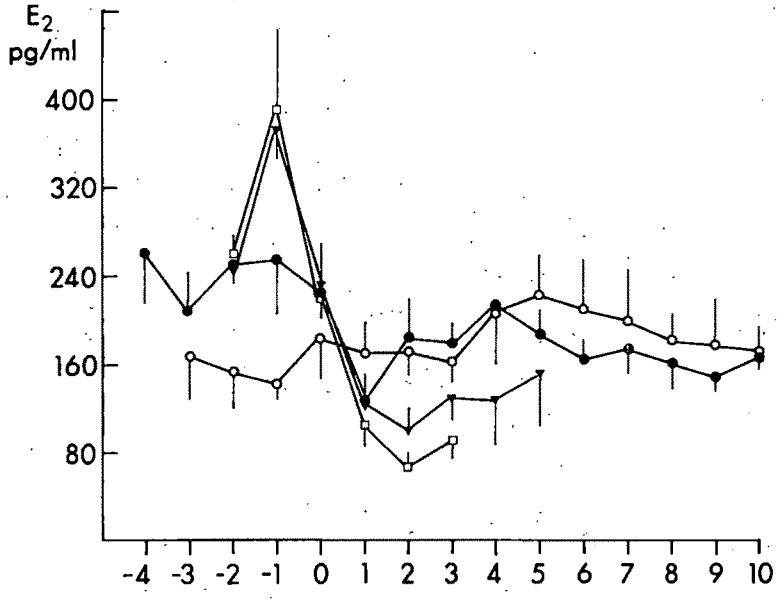


Fig. 5. E_2 values normalized to the day of the LH surge during the pretreatment month (▼), the attenuated (○) and normal (●) surges after RU 486 treatment, and the posttreatment cycle (□). E_2 values on days −2, −1, and 0 were significantly less ($p < 0.005$) during the attenuated response than at any other time.

ity of RU 486 to suppress the midcycle LH/FSH surge and support its development as a contraceptive agent. However, whether the mechanism for this action of RU 486 is primarily due to its suppressive effect on the ovary[11, 15, 16] or to progesterone antagonism at the hypothalamic-pituitary axis will require further study.

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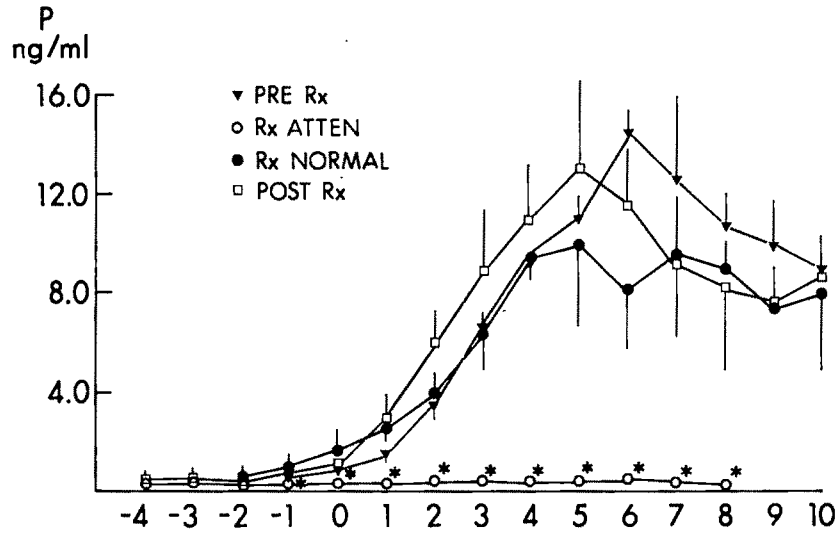


Fig. 6. Progesterone (*P*) values normalized to the day of the LH surge during the pretreatment month (▼), the attenuated (○) and normal (●) surges after RU 486 treatment, and the posttreatment cycle (□). Progesterone levels from days −1 and 0 during the attenuated response were significantly lower ($p < 0.01$) compared with pretreatment and posttreatment values. Progesterone levels from days 1 through 8 after the attenuated LH surge were also significantly lower ($p < 0.01$) than pretreatment values.

Table I. Effect of RU 486 on follicular and luteal phases of the normal menstrual cycle

	Month 1	*Month 2* (treatment month)*	*Month 3*
Total length of cycle	28.8 ± 0.7	40.6 ± 2.6†	25.2 ± 0.9
Follicular phase	14.6 ± 1.3	28.0 ± 2.3‡	13.5 ± 1.9
Luteal phase	15.2 ± 0.8	13.8 ± 1.2	13.5 ± 4.0

Data are $\bar{x}$ ± SEM.
*Discounting the attenuated LH surge.
†$p < 0.01$.
‡$p < 0.005$.

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Serum CA-125 in the evaluation of benign adnexal cysts

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Because serum CA-125 concentrations are increased in women with endometriosis, our objective was to determine whether CA-125 levels could be used to differentiate endometriotic from nonendometriotic beign cysts. During a 2-year period, all women who had an endometrioma (n = 33) or a nonendometriotic cyst (n = 27) that was ≥4 cm were included. Serum CA-125 levels were measured by an immunoradiometric assay, and the mean concentrations in women with an endometriotic cyst increased as the diameter of the cyst increased. The mean CA-125 concentrations in women with cysts ≥4 cm were 53 ± 2 U/ml in 19 women with an endometriotic cyst, 11 ± 1 U/ml in 20 women with a nonendometriotic cyst, and 15 ± 1 U/ml in 7 women with a nonendometriotic cyst and endometriosis. In the fluid of 10 endometriomas, the mean CA-125 concentration was 290 ± 94 × 10^3 U/ml (range 10 to 900 × 10^3 U/ml). With cysts diameters ≥4 cm, 19 of 19 women with endometriotic cysts, zero of seven women with nonendometriotic cysts and endometriosis, and zero of 20 women with nonendometriotic cysts had CA-125 concentrations ≥20 U/ml (99% upper normal limit). We conclude that serum CA-125 determinations offer an excellent method to differentiate endometriotic from nonendometriotic benign cysts. (Am J Obstet Gynecol 1987;157:1426-8.)

Key words: CA-125, ovarian cysts, endometriosis

Adnexal cysts are frequently encountered during the reproductive years. Because most are nonneoplastic functional ovarian cysts, a period of observation is the customary approach when the cyst is less than 6 to 8 cm.[1,2] However, 10% to 35% of benign cystic adnexal masses may be an endometrioma.[1,3] Because endometriomas are not likely to resolve spontaneously, and may enlarge or rupture during the period of observation, a test to detect an endometrioma would assist in the evaluation and management of adnexal cysts. Since serum CA-125 concentrations are elevated in endometriosis,[4,5] we evaluated the serum test to determine whether CA-125 levels could differentiate endometriotic from nonendometriotic cysts.

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Material and methods

Preoperative serum samples were obtained from 60 women of reproductive age (median 28 years; range 13 to 42 years) between 7 to 10 days after the last menses.[6] The women were undergoing laparoscopy or laparotomy for evaluation of a cystic adenxal mass (n = 19) or infertility (n = 41), and informed consent was obtained for evaluation of serum CA-125. When the type of cyst was in question at laparoscopy, a 1 cm biopsy specimen of the cyst wall was obtained for histologic diagnosis. The size of the cysts was determined by direct comparison with a probe calibrated in centimeters. All women with an endometrioma or a nonendometriotic cyst that was ≥4 cm were included. In women (n = 5) who had more than one endometrioma, the diameters were added and the total diameter was correlated with the CA-125 concentration.

CA-125 was measured in the serum samples and endometriotic cyst fluid by an immunoradiometric assay (Centocor, Malvern, Penn.) as previously described.[5] Fisher's exact test and Student's t test with a log transformation when necessary were used to analyze the data.

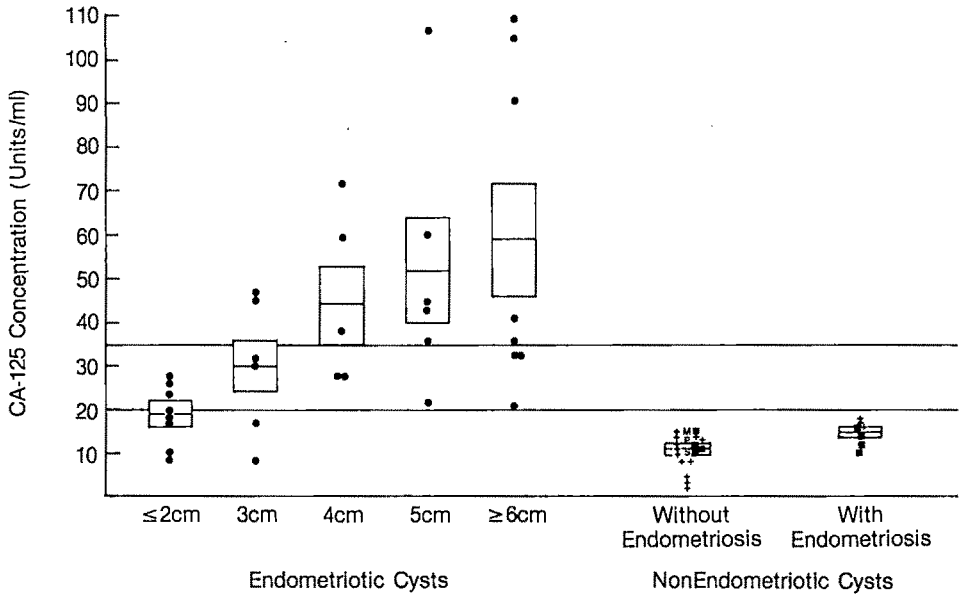


Fig. 1. Serum CA-125 concentrations in endometriotic cysts (by diameter) and nonendometriotic cysts (≥4 cm). The nonendometriotic cysts include follicular (+), corpus luteum (■), serous (*S*), mucinous (*M*), and paratubal (*P*).

Results

Serum CA-125 concentrations increased with increasing size of the endometriotic cyst (Fig. 1). The mean (±SEM) CA-125 concentrations by the total diameter of the endometriotic cyst were: ≤2 cm, 19 ± 3 U/ml; 3 cm, 30 ± 6 U/ml; 4 cm, 44 ± 9 U/ml; 6 cm, 52 ± 2 U/ml; and ≥6 cm, 59 ± 13 U/ml. The types of nonendometriotic cysts included serous (one), mucinous (one), paratubal (one), corpus luteum (nine), and follicular (15); the corresponding serum CA-125 levels for nonendometriotic cysts and nonendometriotic cysts with endometriosis are also shown in Fig. 1. The mean (±SEM) CA-125 concentrations in women with cysts ≥4 cm were 53 ± 12 U/ml in 19 women with endometriotic cysts, 11 ± 1 U/ml in 20 women with nonendometriotic cysts, and 15 ± 1 U/ml in seven women with nonendometriotic cysts and endometriosis. When the fluid in 10 of the endometriotic cysts was analyzed for CA-125, the mean (±SEM) level was 290 ± 94 × 10^3 U/ml (range 10 to 900 × 10^3 U/ml).

With cyst diameters ≥4 cm (Table I), all 19 women with endometriotic cysts, zero of 20 women with nonendometriotic cysts, and zero of seven women with nonendometriotic cysts and endometriosis had serum CA-125 concentrations ≥20 U/ml, which is the 99% upper limit of normal in our assay. In addition, 13 of 19 women (60%) with endometriotic cysts that were ≥4 cm had CA-125 concentrations >35 U/ml, and five of 19 women (26%) had concentrations >65 U/ml.

Table I. Serum CA-125 concentrations in women with benign adnexal cysts ≥4 cm

	CA-125 (U/ml)	
Type of cyst	*<20*	*≧20**
Endometrioma	0	19†
Nonendometriotic	20	0
Nonendometriotic with endometriosis	7	0

*99% Upper normal limit.
†Significantly different, Fisher's exact test ($p < 0.00001$).

Comment

Endometriotic cysts have been shown to be a source of elevations in serum CA-125 levels. This finding has two implications in the clinical management of a woman with an adnexal cyst. First, women with endometriotic cysts ≥4 cm have serum levels of CA-125 that are often >35 U/ml. Because CA-125 concentrations > 35 U/ml suggest epithelial ovarian cancer for which the serum test was developed,[7,8] endometriotic cysts are a significant source of false-positive determinations in the preoperative screening for epithelial ovarian cancer, and vice versa. In one study investigating adnexal cystic masses, approximately 10% of the women had an endometrioma and about 2% had ovarian cancer.[5] Second, in women with adnexal cysts ≥4 cm, serum CA-125 concentrations <20 U/ml are compatible with

a nonendometriotic cyst. Consequently, serum CA-125 determinations offer an excellent method to differentiate endometriotic from benign nonendometriotic cysts. In the young woman of reproductive age with a cystic adnexal mass, a CA-125 level <20 U/ml would support a period of expectant management, and with levels >20 U/ml and particularly >35 U/ml, the possibility of an endometrioma, and at the higher level the possibility of an endometrioma or epithelial ovarian cancer, would require further diagnostic investigation by laparoscopy or laparotomy.

Although the ability to differentiate an endometriotic cyst from benign nonendometriotic cysts was 100% in this study at the 20 U/ml level, it is anticipated that false-positive results with respect to endometriotic cysts will occur. We have observed CA-125 levels ≥20 U/ml in women with endometriosis without an endometrioma,[5] and it would be possible for a similar patient to have a nonendometriotic cyst. Serum CA-125 levels are increased and roughly double during menses,[6] so that care should be taken to avoid obtaining blood samples during menstruation. Also, approximately 9% of women with chronic pelvic inflammatory disease have CA-125 levels >20 U/ml,[5] so that a hydrosalpinx or a tubo-ovarian complex may occasionally have associated elevated levels. Furthermore, early pregnancy and acute pelvic infections frequently result in elevated CA-125 concentrations,[5] although these conditions most often can be differentiated on a clinical and laboratory basis.

We have demonstrated that the use of serum CA-125 level determinations can provide useful information in the evaluation of women with an adnexal cyst. Elevations in CA-125 levels would support proceeding with a laparoscopy or laparotomy to diagnose, stage, and individualize therapy for either endometriosis or, more rarely, ovarian cancer. On the other hand, a CA-125 level <20 U/ml provides reassurance that observation of the cyst is appropriate, and that an endometriotic cyst that may enlarge or rupture during the period of observation is unlikely.

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HLA antigen expression and induction by γ-interferon in cultured human trophoblasts

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The expression and regulation of HLA antigens were examined in isolated human trophoblasts. Immunocytochemical studies that used monoclonal antibodies against class I (HLA-A, B, and C) antigens revealed that both cytotrophoblasts and syncytiotrophoblasts can express HLA heavy chains and β_2-microglobulin. Expression of these antigens increased with time in culture. The addition of recombinant γ-interferon (1000 U/ml) to the cultures significantly increased cellular staining for these antigens over controls throughout the 72-hour time course studied. In addition, we noted that gene expression for HLA class I heavy chain was also markedly augmented by the addition of γ-interferon. Expression of class II (HLA-DR) antigens was not detected in any of the experiments. These results suggest that during in vitro differentiation, cytotrophoblasts can express class I HLA antigens and the genes controlling their production can be regulated. Alterations in the expression or suppression of these antigens during pregnancy could be responsible for some pregnancy complications. (Am J Obstet Gynecol 1987;157:1429-34.)

Key words: HLA antigen, γ-interferon, human trophoblasts, major histocompatibility complex

The products of conception represent a tissue allograft that normally escapes rejection. One approach to explain this phenomemon has focused on the altered expression of major histocompatibility complex antigens on fetal and chorionic tissues. The two categories of major histocompatibility complex antigens most important for immune responses are class I, which are critical as recognition sites for cytotoxic T-lymphocytes, and class II, which are intimately involved in immunoregulation. For humans, class I antigens are the HLA-A, B, and C molecules, which are invariably associated with the monomorphic light-chain, β_2-microglobulin. Class II antigens are the HLA-DR, DP, and DQ molecules. Major histocompatibility complex antigens do not appear to be present on villous syncytiotrophoblasts or cytotrophoblasts in intact villous placental tissue.[1-6] In contrast, some class I antigens have been detected in nonvillous trophoblasts cells[2] and early proliferating cytotrophoblasts.[3] However, there has been a fairly uniform failure to demonstrate the presence of class II antigens such as DR in any trophoblast population.[4-6] Clinically we know that the mother is exposed to these antigens at some point in gestation because of the presence of anti-HLA antibodies in some primigravid and many multiparous women and by the demonstration of anti-idiotypic antibodies in normal pregnant women.[7] Further evidence that these antigens could be expressed in gestation is derived from studies of HLA gene regulation in trophoblast-lineage cells. Genes coding from major histocompatibility complex antigens appear to remain accessible and hence regulatable. Specifically, γ-interferon has been shown to enhance expression of class I major histocompatibility complex antigens in choriocarcinoma cells[8] and human amnion cells.[9]

Kliman et al.[10] recently described a technique of isolating cytotrophoblasts from term human placentas. In culture these cells aggregate and fuse to form syncytiotrophoblasts and exhibit endocrine functions typical of this multinucleated cell layer (e.g., production of human chorionic gonadotrophin, progesterone, and human placental lactogen). Electron microscopic and immunocytochemical studies have shown that the freshly isolated cells represent at least a 95% pure population of cytotrophoblasts.[10] In addition, Main et al.[11] have previously analyzed the surface antigen expression of this population using indirect immunofluorescence and found that 5% express either T cell or macrophage markers.

This in vitro model has already been useful for studying the relationship between differentiation of cytotrophoblasts and endocrine regulation.[12] Therefore we used this culture system to study the immunobiology of trophoblasts. Specifically, we addressed two questions. First, can isolated trophoblasts exhibit major his-

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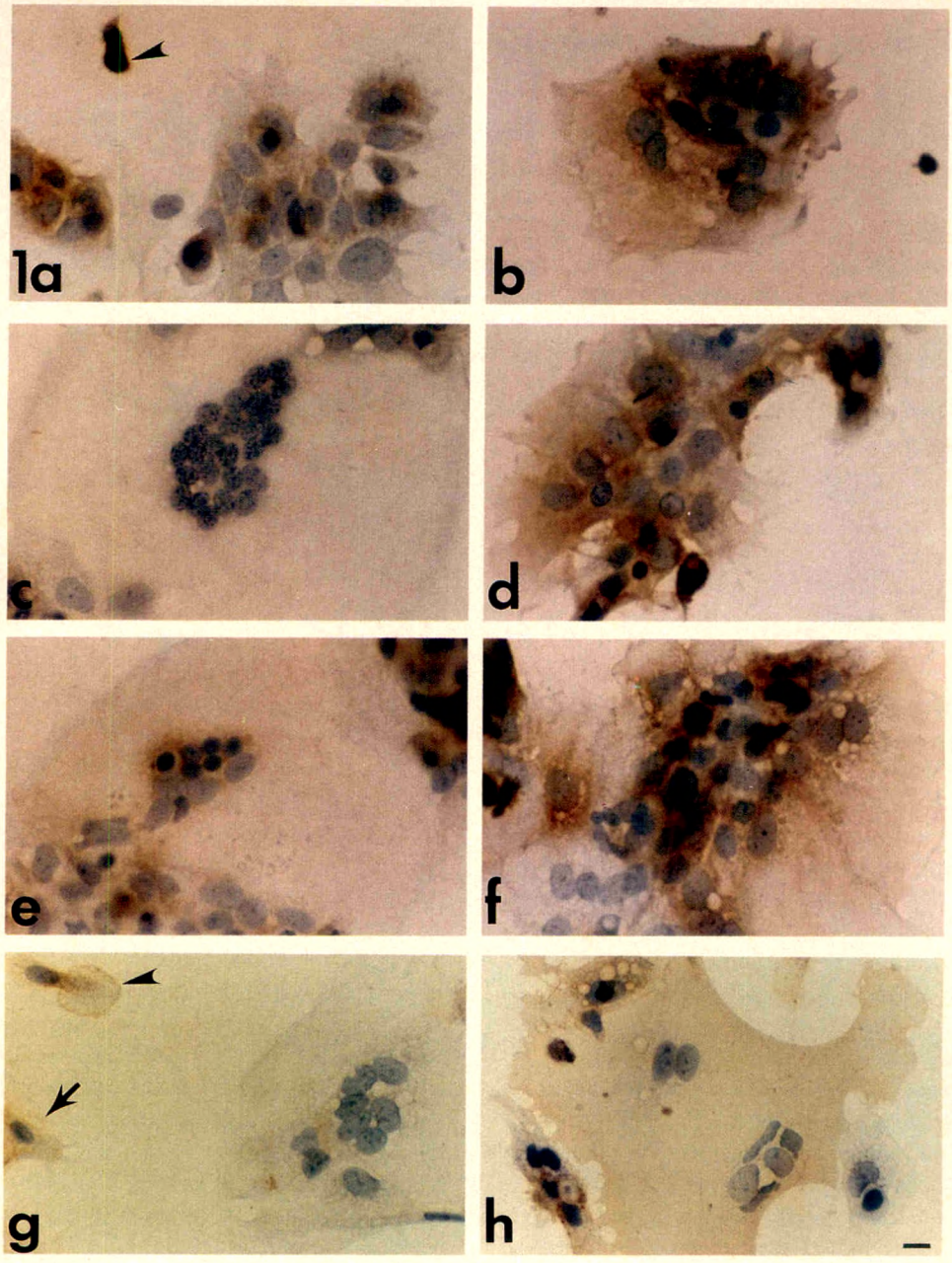


Fig. 1. Immunocytochemical staining of cultured human trophoblasts for β_2-microglobulin, L368 *(a-f)* and class I heavy chain, W6/32 *(g-h)* in the absence *(a, c, e, g)* and presence *(b, d, f, h)* of γ-interferon. Purified human cytotrophoblasts were cultured without (−) or with (+) recombinant human γ-interferon (1000 U/ml) for as long as 72 hours and immunocytochemically stained as described in the Material and Methods section. **a,** 24 Hour −, note light staining for β_2-microglobulin in the trophoblast aggregate whereas the macrophage *(arrowhead)* stained intensely. **b,** 24 hour +, mixed aggregate-syncytia with some cells staining darkly. **c,** 48 hour −, large multinucleated syncytia without staining. **d,** 48 hour +, mixed syncytia-aggregate with lightly and darkly stained cells present. **e,** 72 hour −, large lightly stained syncytia. **f,** 72 hour +, large darkly stained mixed syncytia-aggregate. **g,** 72 hour −, edge of class I heavy-chain reactive trophoblast group *(arrow)* and unreactive large syncytia to the right. Note the positive migrating macrophage *(arrowhead)*. **h,** 72 hour +, large uniformly reactive syncytia. All micrographs are at the same magnification; the *bar* represents 10 μm.

tocompatibility complex antigens in vitro? Second, can expression of these antigens and genes be upregulated by γ-interferon, a known inducer of major histocompatibility complex expression? Because β_2-microglobulin could be expressed with other class I–like molecules, it is important to examine expression of both β_2-microglobulin and HLA heavy chains.

Material and methods

Cytotrophoblasts were isolated from term placentas obtained immediately after delivery. Villous tissue was serially digested with trypsin and deoxyribonuclease, and the resulting cells were placed on a Percoll gradient, as previously described in detail.[10] Suspensions of 0.5 ml containing 500,000 cells were plated on glass coverslips.[10] In some experiments, recombinant human γ-interferon (a generous gift of Genentech, South San Francisco, California) was added to the medium to a final concentration of 1000 U/ml. In some other experiments, 8-bromo-cyclic adenosine monophosphate (Sigma Chemical Co., St. Louis, Missouri) was also added at the time of plating (to a final concentration of 1.5 mmol/L).

Immunocytochemical studies that used an avidin-biotin immunoperoxidase system (Vector Laboratories, Burlingame, California) were performed as described in earlier papers.[10, 13] Monoclonal antibodies to HLA heavy chain (W6/32) and β_2-microglobulin (L368) (both gifts of Dr. L. Lampson, University of Pennsylvania, Philadelphia, Pennsylvania) were used at 1:5 dilutions of hybridoma culture supernatant. Monoclonal antibodies to Dr were used at varying dilutions up to a maximum of 1:2 (OKIa, Ortho Pharmaceutical Co.). Rabbit antibodies against the α-subunit of human chorionic gonadotropin were used at 1:1000 dilutions to confirm the morphologic findings of trophoblasts (gift from Drs. Canfield and Birken, Columbia Medical Center, New York). Negative control hybridoma supernatants and nonimmune rabbit serum were used in each experiment as previously described.[10, 11]

To quantitate the results, 500 nuclei were counted in multiple random fields at each separate time point (4, 12, 24, 48, and 72 hours from culture initiation). The proportions of cells present as single cells, aggregates, and syncytia were calculated. In addition, the percentage of each cell type staining for a specific antigen at each time point was also calculated.

Ribonucleic acid (RNA) was isolated by suspending cultured cells in 4 mol/L guanidinium isothiocyanate followed by ultracentrifugation over a cesium chloride cushion. The 10 μg of each sample underwent electrophoresis through a 1% agarose gel containing 1X MOPS buffer, 6% formaldehyde, and 0.5 μg/ml of ethidium bromide. Using standard Northern blot procedure, the RNA was transferred to Gene Screen Plus membrane (Dupont). After baking in vacuo, the membrane was prehybridized overnight in a solution containing 50% formamide, 1 mol/L sodium chloride, 50 mmol/L Tris, pH 7.4, 1% sodium dodecyl sulfate, 0.5 μg/ml of heparin, 1X Denhardt's solution, 250 μg/ml of salmon sperm deoxyribonucleic acid (DNA), and 10% dextran sulfate. Hybridization with a ^{32}P–Nick-translated cDNA probe for HLA heavy chain (courtesy of S. Weissman, Yale University)[14] was performed in the same buffer for 24 hours. Filters were then exposed for 3 to 24 hours with Kodak XRP film. Scanning densitometry was performed with a LKB ultrascan.

Statistical analysis of the data used χ^2 analysis for comparing the proportions of cells with positive antibody staining at each time point with and without recombinant human γ-interferon treatment. For analysis of change in HLA antigen expression over the time course in culture, we used the Mann-Whitney U test.[15] All studies were conducted with full Human Studies Committee approval.

Results

Under our in vitro conditions, human trophoblasts can express the class I antigens β_2-microglobulin and HLA heavy chain (W/632) (Fig. 1). Immunocytochemical localization of these class I antigens demonstrated that cultured trophoblasts express these proteins between 24 and 72 hours of culture and that recombinant human γ-interferon induced an increased expression of these molecules. In contrast, immunocytochemistry failed to demonstrate staining for Ia in these cultured trophoblasts whether in the presence or absence of recombinant human γ-interferon (data not shown). The few macrophages present (usually under 2% and always under 5%) in these preparations served as internal positive controls for the antibodies examined (Fig. 1, *a* and *g*).

Quantitation of the immunocytochemical studies yielded the results shown in Figs. 2 and 3. Fig. 2 shows the results of immunocytochemical staining for HLA heavy chains (W6/32). In *panel A*, we note the proportion of each cell type present in culture over time. As previously seen, a shift from single cells to syncytia occurred over 72 hours.[10] At 72 hours, the major cell form was the syncytiotrophoblast. The normal morphologic differentiation of the trophoblasts was not affected by recombinant human γ-interferon. Therefore these results represent the average of both the recombinant human γ-interferon treated and untreated cells. We examined the expression over time of HLA heavy chains in the absence or presence of recombinant hu-

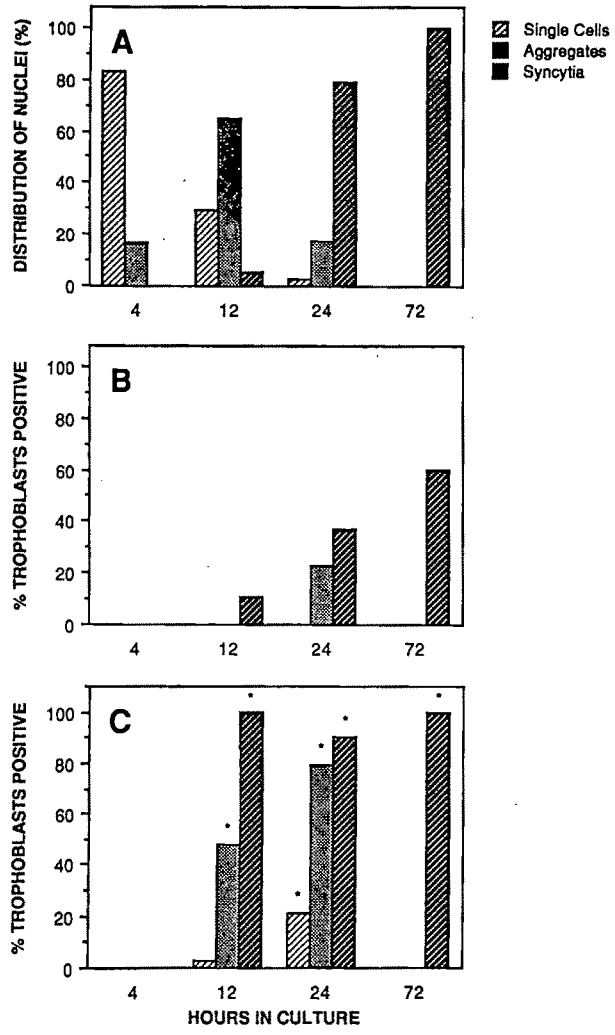


Fig. 2. Quantification of trophoblast morphology **(A)** and immunocytochemical staining for HLA heavy chain (W6/32) in the absence **(B)** and presence **(C)** of γ-interferon. Purified human cytotrophoblasts were cultured for as long as 72 hours as described in the Material and Methods section. **A,** Five hundred nuclei were counted at each time point, and the percentage of single cells, aggregates, and syncytia were calculated. At 4 hours the dominant cell type was the single cell. Aggregates were dominant at 12 hours, whereas syncytia were most prevalent at 72 hours. Treatment with γ-interferon did not affect this progression. **B,** In the absence of γ-interferon, expression of class I heavy chains was first noted at 12 hours in syncytia. At 24 hours, both syncytia and aggregates showed staining. At 72 hours, 60% of the trophoblasts (now all syncytia) stained for the antigen. **C,** In the presence of 1000 U/ml of recombinant human γ-interferon, there was a significant ($p < 0.001$, denoted by *asterisk*) increase in staining for all cell types at all times except for single cells at 12 hours. As shown in Fig. 1, *g-h,* the intensity of the cellular staining also increased.

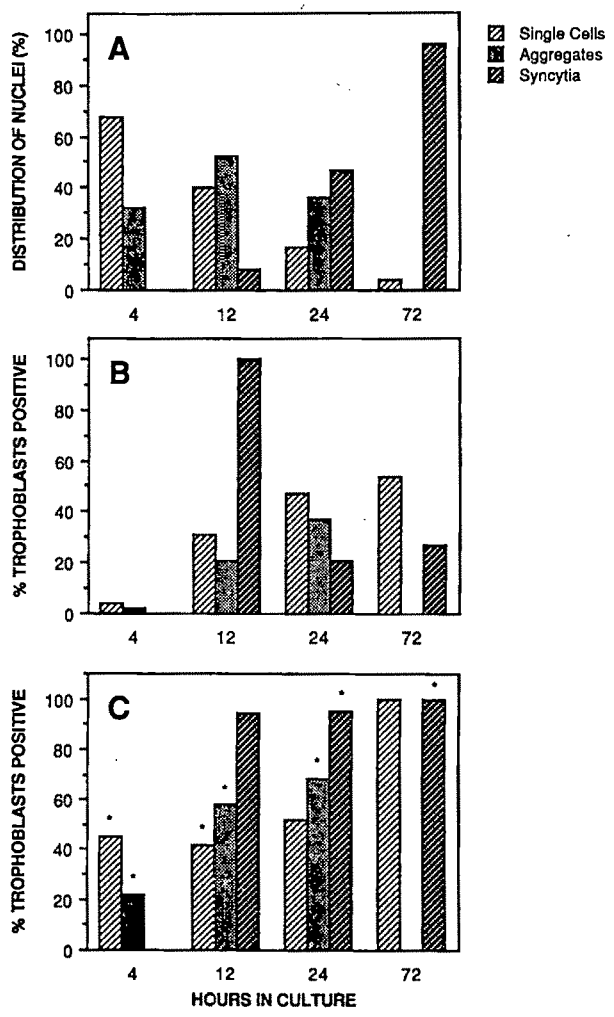


Fig. 3. Quantification of trophoblast morphology **(A)** and immunocytochemical staining for β_2-microglobulin (L368) in the absence **(B)** and presence **(C)** of γ-interferon. Purified human cytotrophoblasts were cultured for as long as 72 hours as described in the Material and Methods section. **A,** Trophoblast forms were calculated as described in the legend to Fig. 2. The progression of trophoblast forms was quite similar to that in Fig. 2. **B,** In the absence of γ-interferon, β_2-microglobulin was detected as early as 4 hours and tended to increase over time. At 72 hours, when syncytia dominated, only 27% of these cells contained detectable levels of β_2-microglobulin. **C,** In the presence of 1000 U/ml of recombinant human γ-interferon, there was a significant ($p < 0.001$, denoted by *asterisk*) increase in the percentage of cells staining for β_2-microglobulin at all time points examined (except for syncytia at 12 hours and single cells at 24 and 72 hours where these forms made up only 8%, 17%, and 4% of the trophoblasts, respectively). In addition to an increase in the percentage of cells staining, γ-interferon induced an increase in intensity of cell staining (Fig. 1, *a-f*).

man γ-interferon. Fig. 2, *B* demonstrates the production of these proteins in the control (untreated) cells. By 12 hours, a few aggregates exhibited the HLA heavy chains. During the next 36 hours, there was a gradual increase in appearance of these molecules, so that at 72 hours, 60% of syncytiotrophoblasts showed light cytoplasmic staining by the antibody. This change over time was significant at the $p < 0.0001$ level. Expression of HLA heavy chains was greatly increased by the ad-

dition of recombinant human γ-interferon, as shown in Fig. 2, *C*. Virtually all the syncytia were strongly stained after 12 hours. Aggregates showed a significant increase in percentage staining at 12 and 24 hours. Even though only a few single cells were counted at 24 hours, there was a significant increase in percentage staining. Significant ($p < 0.001$, χ^2) increases in percentage staining is denoted by an *asterisk* above the bars in Figs. 2 and 3.

Similar experiments were performed that examined the expression of β_2-microglobulin. Fig. 3, *A* shows the proportion of each cell type present at each time point. Fig. 2, *B* shows the fraction of each cell type staining for β_2-microglobulin in the control group. Although a few positive cells were seen at 4 hours, significant staining was first seen at 12 hours. Roughly 20% to 40% of the cells had the molecule. As shown in Fig. 3, *C*, recombinant human γ-interferon greatly enhanced the expression of β_2-microglobulin. Changes were not significant in instances in which few cells were present of a certain type. These experiments were repeated on three occasions, of which all produced similar results. In contrast to its effects on trophoblast hormone production, the addition of 8-bromo-cyclic adenosine monophosphate did not affect the expression of either HLA heavy chains or β_2-microglobulin (data not shown).

Fig. 4 represents a Northern transfer blot demonstrating the presence of class I heavy chain mRNA in unstimulated trophoblasts after 48 hours of culture. Treatment with recombinant human γ-interferon for 24 hours appeared to augment expression of the HLA message. These findings have been replicated three times.

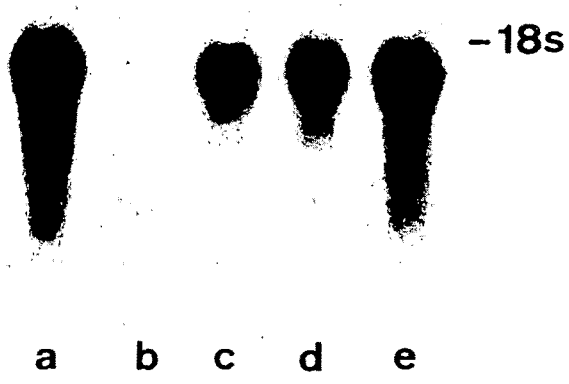


Fig. 4. The γ-interferon augments trophoblast expression of class I heavy-chain mRNA. Isolated mRNA from 8866 (lane a), K562 (lane b), trophoblasts without interferon (lane c), trophoblasts with 4 hours of treatment (lane d), and trophoblasts with 24 hours of 1000 U/ml recombinant human γ-interferon treatment (lane e) underwent electrophoresis, Northern transfer, and hybridization with a ^{32}P–labeled cDNA probe for class I heavy-chain as described in the Material and Methods section. The 8866, a B cell line, serves as the positive control whereas K562, an erythroleukemic line, is the negative control. All the trophoblasts were harvested at 48 hours after culture initiation. By scanning densitometry, lane e (24-hour treatment) represents a 1.8-fold increase over lanes c and d.

Comment

Using a purified cell culture of isolated cytotrophoblasts, we have shown that during differentiation, trophoblasts can express class I major histocompatibility complex molecules. As in other studies, we could not demonstrate expression of class II antigens. The addition of recombinant human γ-interferon greatly enhanced the expression of class I major histocompatibility complex antigens both in terms of intensity of staining and the number of cells staining for HLA heavy chain (W6/32) and β_2-microglobulin (L368). Furthermore, RNA analysis confirms these immunohistochemical findings by demonstrating measurable class I mRNA in unstimulated trophoblasts cultured for 48 hours and induction of this mRNA in the presence of recombinant human γ-interferon. Thus the genes coding for class I molecules are not irreversibly downregulated in isolated trophoblasts. These results imply that control of HLA expression in intact villi may be by another process.

Recently, Hunt et al.[16] reported an inability to induce HLA antigen expression by purified γ-interferon in tissue explants derived from first- and third-trimester placentas.[16] There are several reasonable explanations for the differing results between these findings and ours. First, Hunt et al. examined tissue explants with intact villi that may possess the ability to downregulate HLA expression. We used cytotrophoblasts removed from the chorionic villi, thus possibly freeing them from this inhibition. Second, by definition, any placental explant experiments use well-differentiated syncytiotrophoblasts. Our cell culture system allows us to study cytotrophoblasts as they differentiate in culture. The expression of HLA antigens may occur very early in gestation as syncytia form.

We have previously demonstrated that cytotrophoblasts in culture functionally differentiate and assume endocrine functions typical of syncytiotrophoblasts. This process is enhanced by 8-bromo-cyclic adenosine monophosphate.[12] In contrast, the addition of

8-bromo-cyclic adenosine monophosphate did not affect expression of HLA antigens.

These results suggest that differentiating trophoblasts are capable of expressing class I major histocompatibility complex antigens. The following are several possible explanations for the absence of these proteins in the intact placenta: (1) The production of these antigens may be suppressed in normal pregnancy by local or maternal factors; (2) the antigens may be lost as the villous syncytia mature; or alternatively, (3) the cells may produce HLA antigens, but are induced to shed them before or as soon as they are incorporated into the membrane.

Since recombinant human γ-interferon enhanced expression of class I major histocompatibility complex antigens, it may be useful to see if there is an increased expression of these proteins in pathologic conditions. This could explain the possible but unproved relationship between infections and habitual abortions. Potentially, interference with mechanisms that downregulate the expression of these antigens may also account for other pregnancy complications.

Finally, we feel that the purified cell culture system used in this study will prove to be a useful model to help us understand how the products of conception attain their immunologically protected status. Contrasting results between studies that use cell cultures and intact tissue preparations could help identify which factors play a role in this important process.

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Cerebrospinal fluid levels of magnesium in patients with preeclampsia after treatment with intravenous magnesium sulfate: A preliminary report

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The purpose of this study is twofold: (1) to correlate magnesium levels of serum with those of cerebrospinal fluid in patients with preeclampsia receiving intravenous magnesium sulfate, and (2) to determine whether the magnesium ion crosses the blood-brain barrier in significant amounts after intravenous magnesium sulfate therapy. Of the 21 patients in whom spinal anesthesia was used for delivery, ten patients with preeclampsia with therapeutic serum magnesium levels made up the study group and 11 term nontreated normotensive gravid women served as controls. At the time of spinal anesthesia, a 1 ml aliquot of cerebrospinal fluid was obtained from each patient. The mean cerebrospinal fluid magnesium level for the control group was 2.56 ± 0.19 mg/dl (range 2.2 to 2.8 mg/dl). For the preeclamptic group who received intravenous magnesium sulfate, the mean cerebrospinal fluid magnesium level was 3.04 ± 0.12 mg/dl (range 2.9 to 3.2 mg/dl). Although only a small amount of magnesium crosses the blood-brain barrier after intravenous magnesium sulfate treatment, this increment is highly significant ($p < 0.001$). (Am J Obstet Gynecol 1987;157:1435-8.)

Key words: Cerebrospinal fluid, magnesium sulfate, preeclampsia, blood-brain barrier

The incidence of eclamptic seizures is extremely low after parenteral magnesium sulfate ($MgSO_4$) therapy of the patient with preeclampsia.[1, 2] However, the unresolved question is: Does magnesium (Mg^{++}) cross the blood-brain barrier in significant amounts and provide a central nervous system site of action after parenteral $MgSO_4$ therapy? Despite previous studies[3-6] on this issue, controversy continues with regard to the site of action of $MgSO_4$ in the prevention and treatment of eclamptic convulsions.[7, 8] Furthermore, no investigator has proved that the small increment in cerebrospinal fluid Mg^{++} levels after intravenous $MgSO_4$ treatment of preeclampsia-eclampsia is either statistically significant or related to dose and time.

The purpose of our study is twofold: (1) to correlate serum magnesium levels with those of cerebrospinal fluid in patients with preeclampsia who receive $MgSO_4$, and (2) to determine whether Mg^{++} crosses the blood-brain barrier in significant amounts after intravenous $MgSO_4$ therapy.

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Methods

The sample population consisted of 21 gravid women in whom spinal anesthesia was used for delivery. Eleven normotensive gravid women served as controls, of which nine were delivered by cesarean section and two by low forceps after saddle blocks. The study group consisted of ten gravid women with preeclampsia according to classic criteria[9] for whom intravenous $MgSO_4$ treatment was started at least 4½ hours before delivery (range 4½ to 72 hours). Of these, five were delivered by cesarean section and five were delivered by low forceps after saddle blocks.

Patients without a contraindication to spinal anesthesia were prospectively entered into the study after giving informed consent. For those patients with preeclampsia, intravenous $MgSO_4$ was administered in the following manner: a 6 gm loading dose was given over 15 to 20 minutes, followed by a 2 gm/hr maintenance dose.

In all patients, a lumbar puncture was performed with a 25 gauge 3½ inch needle and cerebrospinal fluid flow was confirmed in all four quadrants. A 1 ml aliquot was then obtained by dependent drainage, after which Xylocaine spinal anesthesia was administered. Simultaneously, a venipuncture was performed to acquire a 3 ml aliquot of serum for Mg^{++} assay.

Assays of cerebrospinal fluid and serum Mg^{++} were performed by means of an automatic clinical analyzer magnesium method, which is a modification of the methylthymol blue complexometric procedure.[10]

Table I. Descriptive and outcome variables for individual patients

Group	Age (yr)	Parity	Gestational age (wk)	Time between Mg^{++} treatment and delivery	Mg^{++} levels (mg/dl)		Delivery outcome
					Serum	CSF	
Control							
1	18	1001	39	0:00	1.2	2.3	C/S
2	17	0000	40	0:00	1.3	2.5	C/S
3	24	1001	38	0:00	1.3	2.6	C/S
4	26	4014	42	0:00	1.4	2.2	Vag
5	22	1314	38	0:00	1.4	2.5	C/S
6	27	2002	38	0:00	1.4	2.5	C/S
7	22	1102	38	0:00	1.4	2.6	Vag
8	24	1001	39	0:00	1.5	2.7	C/S
9	25	1011	37	0:00	1.5	2.8	C/S
10	31	1011	37	0:00	1.6	2.7	C/S
11	21	0010	43	0:00	1.8	2.8	C/S
Patients with preeclampsia							
1	19	0000	39	4:30	4.2	2.9	C/S
2	18	0010	40	4:30	4.3	2.9	Vag
3	30	0000	38	4:30	4.8	2.9	Vag
4	18	0000	40	4:30	5.2	3.0	C/S
5	20	0010	38	6:00	5.9	3.1	Vag
6	16	0000	38	6:45	5.9	3.0	Vag
7	19	0000	42	8:30	6.8	3.2	C/S
8	22	0201	28	22:00	5.8	3.1	Vag
9	20	0010	35	25:00	6.2	3.1	C/S
10	18	0000	38	72:00	6.2	3.2	C/S

CSF, Cerebrospinal fluid; *C/S*, cesarean section; Vag, vaginal delivery.

Table II. Results of study

Group	N	Magnesium level (mg/dl)	
		Serum	CSF
Control	11		
Mean ± SD		1.44 ± 0.16	2.56 ± 0.19
Range		1.2-1.8	2.2-2.8
Patients with preeclampsia	10		
Mean ± SD		5.53 ± 0.87	3.04 ± 0.12
Range		4.2-6.8	2.9-3.2
t test (*p* value)		<0.001	<0.001

CSF, Cerebrospinal fluid.

Results

Individual descriptive and outcome variables for the sample population are given in Table I.

The mean (±SD) serum Mg^{++} level for the control group was 1.44 ± 0.16 mg/dl (range 1.2 to 1.8 mg/dl). For the preeclamptic group, the mean serum Mg^{++} level was 5.53 ± 0.87 mg/dl (range 4.2 to 6.8 mg/dl). As shown in Table II, the mean serum Mg^{++} level of the group receiving intravenous $MgSO_4$ is significantly higher than that of the untreated control group ($p < 0.001$).

The mean cerebrospinal fluid (±SD) magnesium level for the control group was 2.56 ± 0.19 mg/dl (range 2.2 to 2.8 mg/dl). For the group with preeclampsia who received intravenous $MgSO_4$, the mean cerebrospinal fluid Mg^{++} level was 3.04 ± 0.12 mg/dl (range 2.9 to 3.2 mg/dl). When compared with the untreated control group, the mean cerebrospinal fluid Mg^{++} level was significantly higher in the group treated with intravenous $MgSO_4$ ($p < 0.001$). Furthermore, no overlap of the cerebrospinal fluid Mg^{++} levels was observed between the two groups (Table II).

Next, CSF Mg^{++} levels *(y)* were plotted as a function of serum Mg^{++} levels *(x)* and regression analysis was performed (Fig. 1). Regression analysis of the control group produced the following regression equation: $y = 1.40 + 0.808x$ ($r = 0.689$). Regression analysis of the group treated with intravenous $MgSO_4$ produced the following regression equation: $y = 2.35 + 0.125x$ ($r = 0.925$). Although the regression lines for both groups in Fig. 1 are extended beyond the range of their respective values, theoretic extrapolations are not implied.

Comment

In 1955 Pritchard[3] evaluated magnesium levels in plasma and cerebrospinal fluid of 10 preeclamptic-eclamptic patients treated with intramuscular $MgSO_4$. Although he found cerebrospinal fluid Mg^{++} levels to be slightly higher in the $MgSO_4$-treated group (2.65 versus 2.35 mEq/L), he made two conclusions: (1) an equilibrium between plasma Mg^{++} (3.0 to 8.0 mEq/L for 3 to 48 hours) and cerebrospinal fluid Mg^{++} (average = 2.65 mEq/L) was never obtained, and (2) the cerebrospinal fluid level of Mg^{++} is not dependent on the plasma level. Although plasma and cerebrospinal fluid magnesium levels in our patients are similar to

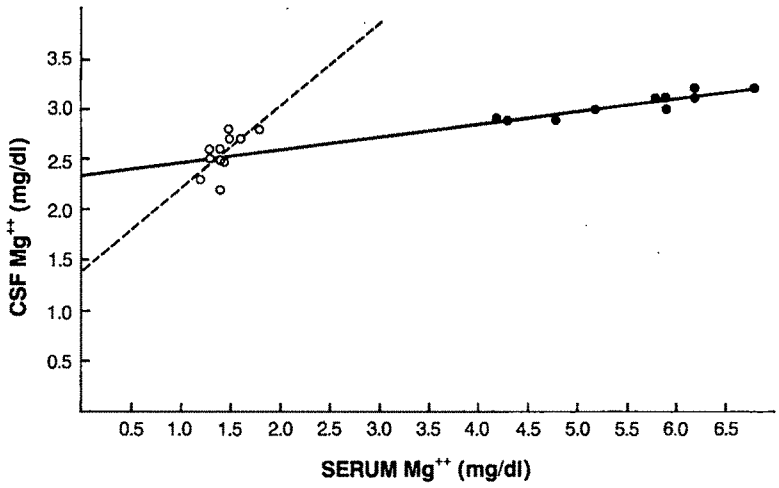


Fig. 1. Relationship between cerebrospinal fluid (*CSF*; y) and serum (x) Mg^{++} levels. *Open circles* represent the nontreated normotensive control subjects, and *closed circles* represent the preeclamptic gravid women who received intravenous $MgSO_4$. Regression equations: Controls, $y = 1.40 + 0.808x$ ($r = 0.689$); patients with preeclampsia, $y = 2.35 + 0.125x$ ($r = 0.925$).

those of Pritchard's, our conclusions are opposite of his. Statistical analysis of the small but statistically significant increment of cerebrospinal fluid Mg^{++} in the treated group may be the explanation.

Oppelt[4] in 1963 evaluated Mg^{++} levels in plasma and cerebrospinal fluid of 29 mongrel dogs treated with intravenous $MgSO_4$. He found that Mg^{++} crossed the blood-brain barrier in small but significant amounts, and speculated that it may be influenced by an active transport mechanism. He concluded that the appearance of a significant increase of Mg^{++} in the cerebrospinal fluid may be a time-related phenomenon.

Somjen[5] in 1968 investigated the ability of intrathecally administered Mg^{++} to depress excited target neurons in the cerebral cortex of 11 cats anesthetized with pentobarbital sodium. He demonstrated that Mg^{++} significantly reduces the electrical discharge frequency in cortical neurons. He concluded that a relatively small increment in cerebrospinal fluid Mg^{++} concentration has a significant depressant effect on cortical function.

In 1978 Borges[6] studied the effect of intravenous $MgSO_4$ on the electroencephalographic patterns of 10 anesthetized cats with *n*-penicillamine–induced seizures. As the plasma Mg^{++} levels increased, he observed an associated decrease in cortical firing. He speculated that the degree of central nervous system depression secondary to increased plasma Mg^{++} levels was dose and time dependent.

Despite the findings of these studies, over the past 30 years three questions have remained unresolved: (1) After intravenous $MgSO_4$ therapy, does Mg^{++} cross the blood-brain barrier in significant amounts? (2) Does an increment in cerebrospinal fluid Mg^{++} provide a central nervous system site of action? (3) Is the cerebrospinal fluid Mg^{++} increment observed in $MgSO_4$-treated patients simply correlated to the serum Mg^{++} level, or is it related to dose and time? Because the appearance of a significant increment of Mg^{++} in the cerebrospinal fluid may be a dose- and time-related phenomenon influenced by an active transport mechanism,[4] we designed this preliminary study to evaluate cerebrospinal fluid Mg^{++} levels after at least 4½ hours of intravenous $MgSO_4$ treatment.

Based on these preliminary data, we believe that cerebrospinal fluid Mg^{++} levels *(y)* are highly correlated with serum Mg^{++} levels *(x)* in patients treated with intravenous $MgSO_4$ (regression equation: $y = 2.35 + 0.125x$; $r = 0.925$). Although only a small amount of Mg^{++} crosses the blood-brain barrier after at least 4½ hours of intravenous $MgSO_4$ therapy, we found this increment to be highly statistically significant ($p < 0.001$).

Despite our findings, two questions remain unanswered: (1) What is the minimum $MgSO_4$ dose and minimum treatment interval that will provide a significant cerebrospinal fluid Mg^{++} concentration increment? (2) Does an elevated CSF Mg^{++} level reflect a central nervous system site of action?

To address these two issues, further studies are currently underway. The answers to these two questions may explain Chesley and Tepper's observation of eclamptic seizures that occasionally occur in preeclamptic patients with confirmed "therapeutic serum levels" of Mg^{++} after parenteral $MgSO_4$ therapy.[11]

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Effects of cochlear ablation on local cerebral glucose utilization in fetal sheep

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Local cerebral glucose utilization was measured by the [^{14}C]-deoxyglucose method in five near-term fetal sheep in whom bilateral ablation of the cochleae had been accomplished aseptically 5 to 8 days earlier. The tympanic membrane and ossicles were removed and all turns of each cochlea were unroofed with destruction carried to the modiolus. Mean local cerebral glucose utilization of 33 of 34 gray matter structures and four of four white matter structures in operated animals were significantly lower ($p < 0.05$) than that in unoperated control fetuses. The depression in local cerebral glucose utilization was greatest ($p < 0.002$) in brain stem auditory nuclei, in which the mean rate of glucose utilization was approximately 25% of the levels in unoperated fetuses. The pattern of glucose utilization in these structures was clearly altered, with a reversal of the normal distribution in density of the inferior colliculus. Tonotopic bands of high local cerebral glucose utilization frequently seen in autoradiographs of inferior colliculus in unoperated fetuses were not observed in operated fetuses. These results show that the glucose utilization of the brain, and by implication the normal growth and maturation of the brain, depends on an intact auditory system during prenatal life. (Am J Obstet Gynecol 1987;157:1438-42.)

Key words: Fetal sheep, local cerebral glucose utilization, brain stem, auditory nuclei, cochlear ablation

Environmental sounds are known to penetrate the abdominal wall and uterus of pregnant women. Specific sounds can induce fetal movements[1, 2] and cardioacceleration,[3-5] reduce fetal breathing movements,[1] and evoke fetal electroencephalographic responses.[6] There have been speculations about the importance of sound in prenatal nervous system maturation,[7] imprinting,[8] and early neonatal speech recognition.[9] Fetal cochlear damage may result if noise is excessive.[10] Thus prenatal sound may have far-reaching implications for the developing child.

The constraints placed on human experimentation force us to turn to appropriate animals to further our understanding in this area of developmental bioacoustics. As a model for human characteristics, the pregnant sheep has two advantages over common laboratory animals: (1) the physical dimensions (and thus the acoustic properties) of the ewe and her products of conception, including fetal fluids, are similar to those in human pregnancy, and (2) the cochlea and central auditory connections are anatomically completed and presumed functional by the third trimester of gestation in both species.[11, 12] An important first step in our understanding of this model, the characterization of the fetal sound

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Table I. Data from experiments to measure the effect of bilateral cochlear destruction on local cerebral glucose utilization in fetal sheep

	Cochlear destruction					*Control*				
	126	*122*	*W-82*	*Y-81*	*0-9*	*0-28*	*0-17*	*214*	*0-14*	*0-2*
Gestational age (days)	142	141	135	134	137	135	135	138	137	135
Time after operation (days)	3	3	8	5	5	4	4	7	8	5
Fetal weight (kg)	3.7	3.8	3.3	2.4	—	3.5	—	4.4	3.5	2.5
Glucose, fetus (mg/ml)	0.27	0.56	0.27	0.17	0.14	0.21	0.14	0.27	0.16	0.13
Temperature of ewe (° C)	39.4	39.4	39.4	39.7	—	—	—	—	—	—
Fetal arterial pH*	7.411	7.377	7.370	7.384	7.393	7.380	7.376	7.361	7.370	7.316
	7.431	7.380	7.364	7.384	7.472	7.368	7.349	7.377	7.364	7.330
Fetal Pa_{CO_2} (mm Hg)*	63.5	47.2	60.3	43.5	33.2	42.8	50.6	36.4	37.2	40.9
	59.3	48.8	57.3	44.6	40.2	41.7	54.6	34.2	44.4	40.5
Fetal Pa_{O_2} pressure (mm Hg)*	10.9	15.2	23.1	16.3	16.6	20.6	17.8	16.4	21.3	17.5
	16.2	15.0	28.7	18.4	18.7	19.5	18.8	20.7	22.2	21.0
Fetal hematocrit	39	40	37	34	30	37	32	—	35	43
Rapid eye movement sleep† (%)				43	29	77	16	28	31	34
Non–rapid eye movement sleep† (%)				53	55	18	54	57	69	56

*Control and +20 minutes.

†Weighted for fraction of free deoxyglucose concentration in the tissue integrated with respect to time.

environment within the pregnant ewe, has already been made.[13-15] Thus a 2 to 5 dB enhancement within the ewe for frequencies <0.25 kHz was found; frequencies >0.25 kHz were progressively attenuated up to 20 dB at 4.0 kHz; external speech and music were easily recognizable, albeit muffled, once the noise floor (50 dB spectrum level) was exceeded.

While these results proved that the fetal sound environment is richer and more varied than heretofore believed,[13] the specific effects of sound on the fetus are less accessible to quantitative assessment, and are therefore sometimes inconsistent.[4, 7] One consistent finding, however, is the high rate of glucose utilization along the central auditory pathways of the fetus in utero.[16] The origins of this high metabolic rate for glucose, which is also present in animals after birth,[17-20] has not been established. The present study was undertaken to determine the importance of the cochlea in sustaining this high rate of local cerebral glucose utilization in the fetal sheep.

Methods

Ten grade Western ewes with known dates of insemination were fasted for 24 hours, anesthetized with halothane by mask, intubated, and carried throughout an aseptic surgery with halothane and oxygen. The fetal head was located through the uterine wall and moved to a relatively avascular area on the greater curvature. With the head held firmly by one hand, the uterine serosa, but not underlying tissues, was incised over 4 inches. Small openings in the myometrium and membranes were made, and these tissues were then stretched to the extent of the serosal incision. This procedure resulted in little bleeding. The fetal head and one foreleg were delivered. Polyvinyl catheters were placed in the fetal brachiocephalic artery and superior vena cava through incisions in the axillary artery and a superficial shoulder vein.

In five experimental animals, we used an operating microscope to remove the tympanic membrane and ossicles, working through a postauricular approach. Oval and round windows were identified. With the aid of round cutting burs, all turns of the cochlea were unroofed, with destruction carried to the modiolus.

After completion of three ablation experiments, it was brought to our attention that fetal cerebral oxygen consumption rates and local cerebral glucose utilization rates were correlated with the behavioral state of the fetus at the time of the experiment.[21, 22] Thus in five control animals and two of five experimental animals, stainless steel screws (0-80; 1/8 in) were placed bilaterally in parietal bone 1 to 2 cm on either side of the midline cranial suture. Additional screws (0-80; 1/4 in) were placed through predrilled holes in the roof of the orbit. Stranded tinned copper lead wires, previously silver soldered to the screws, were led out through the scalp and skin incisions. Wire electrodes (Cooner Wire Company, Chatsworth, Calif.) placed on a neck strap muscle completed the instrumentation required for assessment of fetal behavioral state.

All fetal skin incisions were closed. Ampicillin, 0.5 gm, was introduced in the amniotic fluid. The fetal

Table II. Local cerebral glucose utilization (μmol/100 gm/min)

	Cochlear destruction					*Controls*					
	126	*122*	*W-82*	*Y-81*	*0-9*	*0-28*	*0-17*	*0-14*	*214*	*0-2*	*p**
Motor, sensory cortex	20	18	9	15	30	35	29	54	20	48	0.029
Visual cortex	23	20	10	15	24	30	29	46	21	51	0.025
Auditory cortex	17	20	7	13	22	27	22	42	17	47	0.044
Cingulate gyrus	27	22	12	21	33	36	41	66	24	70	0.034
Thalamus	20	18	10	21	35	45	42	68	29	69	0.009
Medial geniculate	24	19	—	21	35	53	62	103	41	96	0.014
Lateral geniculate	37	28	15	35	45	57	55	98	35	78	0.025
Hypothalamus	14	17	7	13	22	30	20	37	18	39	0.021
Mamillary body	52	31	18	39	57	59	51	111	41	112	0.050
Hippocampus	27	18	10	21	31	31	50	44	26	60	0.020
Amygdala	15	12	10	15	30	32	30	64	29	57	0.014
Septum	19	14	9	17	31	45	32	74	20	54	0.027
Caudate	18	14	8	20	30	25	31	47	22	50	0.036
Putamen	18	18	10	17	27	27	35	48	24	52	0.015
Subthalamic nuclei	25	19	21	38	44	69	56	124	34	95	0.022
Vestibular nuclei	20	21	17	38	40	61	76	115	41	100	0.007
Cochlear nuclei	13	18	8	19	29	50	57	92	38	72	0.002
Superior olives	16	19	10	19	30	68	86	105	44	100	0.0007
Inferior olives	24	23	15	35	46	52	75	122	39	91	0.016
Inferior colliculus	24	28	21	34	33	82	105	178	73	126	0.002
Superior colliculus (st. super.)	28	30	15	34	43	38	56	77	30	101	0.058
Superior colliculus (st. profund.)	16	16	10	19	25	29	30	45	18	65	0.045
Habenula	22	18	10	25	44	46	48	85	25	97	0.036
Preoptic area	14	14	9	13	20	27	22	38	19	44	0.014
Spinal cord, ventral horns	13	17	8	21	29	39	48	—	27	43	0.006
Reticular formation	16	14	10	17	27	35	31	48	17	47	0.018
Lateral lemniscus	18	19	10	20	27	50	66	89	39	71	0.001
Periaqueductal gray	13	16	7	12	21	24	18	35	15	35	0.041
Nuclei of oculomotor nerves	22	18	13	29	34	34	49	68	35	72	0.012
Red nuclei	19	18	12	30	40	44	—	64	25	70	0.039
Vermis	19	20	10	16	30	36	51	63	23	50	0.010
Cerebellar cortex	11	12	8	14	21	25	22	36	16	38	0.017
Cerebellar nuclei	24	26	16	32	52	50	61	100	36	82	0.024
Flococulus	20	19	14	22	40	35	48	56	25	62	0.026
Spinal cord, lateral columns	8	11	5	10	15	19	29	—	16	25	0.006
Internal capsule	14	10	7	12	24	20	23	34	14	30	0.046
Corona radiata	9	8	3	7	9	11	22	16	12	13	0.010
Optic chiasm	10	14	3	11	12	14	18	25	16	28	0.014

*Destruction versus controls.

head and foreleg were repositioned in the uterus and the uterus was closed. A second dose of 0.5 gm ampicillin was placed in the peritoneal cavity of the ewe before the anterior abdominal wall was closed. Leads were tunneled subcutaneously and exited on the maternal flank, where they were stored in a pouch sutured to the skin. The skin was closed with clips. Finally, maternal femoral arterial and venous catheters were placed. Anesthetic levels were reduced during the closing procedure so that the ewe stood within 5 minutes of extubation.

Ewes were placed in carts daily to flush catheters. A cart containing a companion ewe was positioned nearby. On the day of the local cerebral glucose utilization experiment (3 to 8 days after surgery), the fetal behavioral state was assessed during a 2-hour control period (Table I).

Details of the method used to measure cerebral glucose utilization have been published.[16] Briefly, 100 μCi [^{14}C]-2-deoxyglucose (American Radiolabeled Chemicals, St. Louis, Mo.) per kilogram of estimated fetal weight were given over 30 seconds. The histories of the arterial plasma levels of [^{14}C]-2-deoxyglucose and glucose were determined by frequent blood sampling for 30 to 40 minutes. Fetuses and ewes were given intravenous sodium pentobarbital solution followed immediately by intravenous KCl to induce cardiac arrest. The fetus was delivered and weighed and the brain was removed and frozen for later sectioning and quantitative autoradiographic examination.[16]

Local cerebral glucose utilization was calculated for 38 cerebral structures. Means and SEM of values for experimental and control groups were calculated across structures. Comparisons between groups were by

paired Student's t test with significance taken as $p < 0.05$. The average rate of glucose utilization for the entire brain was not measured.

Results

Gross inspection of the fetal temporal bones removed after the study revealed that the cochleae were destroyed bilaterally to the level of the modiolus.

Table I summarizes data for both experimental and control fetuses. Fetal weights, pH, $PaCO_2$, PaO_2, and glucose levels were within the range of those characteristic of our flock.[16] Local cerebral glucose utilization in experimental fetuses was uniformly depressed, with 33 of 34 gray matter structures and four of four white matter structures differing significantly ($p < 0.05$) from respective structures in control fetuses (Table II). Reduction in local cerebral glucose utilization in the four brain stem auditory nuclei (cochlear nuclei, superior olives, lateral lemniscus, and inferior colliculus) reached even higher levels of significance ($p < 0.002$). The pattern of glucose utilization in the inferior colliculus as represented pictorially in autoradiographs was clearly altered, with the appearance of a very pale central nucleus and a denser periphery.

Behavioral state cycling during the 30 to 40 minutes required to measure local cerebral glucose utilization appeared to be normal in two operated fetuses, and the quality of the electrocorticogram, electrooculogram, and neck electromyographic tracings was good.

Comment

The [^{14}C]-2-deoxyglucose method of Sokoloff et al.[23] offers a powerful method of metabolic encephalography. As seen consistently on autoradiographs, the brain structures with the highest concentrations of [^{14}C] (and thus with the highest glucose utilization rates) are the cochlear nuclei, superior olives, lateral lemnisci, and inferior colliculi—all structures in the central auditory pathway. Audiographs of the inferior colliculi are characterized by differing densities, with the darkest area occupying part or all of the central nucleus. This dark region, whose metabolic rate normally exceeds that of all other loci in the brain, commonly takes on the shape of an ovoid or band. Other bands, presumably representative of a tonotopic, spacial organization of frequency coding,[18-20] are occasionally seen.

These autoradiographic features have been observed by us in all normal fetal sheep. We inferred that the high rates of glucose utilization in central auditory structures could be at least partly related to environmental sound that is continuously present.[13-15] The sound originates outside the ewe as well as within the ewe, through intestinal, cardiovascular, and respiratory activities and through vocalization. Intra-amniotic pressure changes resulting from pulsation of the umbilical artery could be an additional source of sound. Because of the well-known link between local functional activity and local metabolic rate in the brain,[17] ambient noise intense enough to evoke fetal cardioacceleration and movement would be expected to activate the metabolism along the auditory pathways. The specificity of this metabolic response, depending as it must on the maturation of the central auditory pathways and connections, would be more advanced in sheep fetuses than in term fetuses of less precocious species.

Bilateral destruction of the fetal cochleae resulted in a dramatic reduction in glucose utilization in the central auditory nuclei. This phenomenon has been well documented postnatally in animals.[17, 24, 25] We did not explore the effects of unilateral destruction, but this procedure has been used to judge the levels and extent of crossing of afferent auditory fibers.[25]

A decrease in glucose utilization in nonauditory structures in operated fetuses was not anticipated, and has not been mentioned previously in descriptions of cochlear ablation in animals postnatally. Virtually all gray and white matter structures were affected to a significant extent. In earlier experiments in fetal sheep, glucose utilization was found to be relatively high in brain stem auditory structures at the beginning of the third trimester.[16] Thus central auditory projection to nonauditory structures, including the reticular formation, would appear early and could dominate the representation of other sensory modalities that have yet to mature to the same degree. Continuing with this reasoning, removal of the cochleae could result in a diffuse inactivation of the central nervous system, which would be reflected in its lowered metabolic rate. The reduction could be linked to the elimination of any transduction of acoustic energy by the destroyed cochleae as well as to the removal of spontaneous, resting neural discharge of auditory neurons.[20] The possibility must also be considered that the overall reduction in cerebral glucose utilization may have resulted from the generalized trauma associated with the destructive lesioning. However, the technique used in bilateral cochlear destruction was entirely peripheral, without violation of dura or intracranial vasculature or contents. While microcontamination of cerebrospinal fluid via a patent cochlear aqueduct is possible, the effects of such blood contamination must be considered minimal. Whatever the cause of the lowered cerebral metabolic rate, it may have some consequences for normal maturation of the fetal brain.

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Umbilical artery flow velocity waveforms and placental resistance: The effects of embolization of the umbilical circulation

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Embolization of the umbilical placental circulation in fetal lambs was carried out to occlude the small vessels of the placental vascular bed and to observe the effect on the umbilical artery flow and flow velocity waveforms. Thirteen singleton fetuses were studied from day 120 of pregnancy. Embolization was achieved by injecting approximately 9×10^6 microspheres of 15 μm diameter into the fetal placental cotyledons along the umbilical arteries over 9 days. Umbilical and uterine flows were measured by radioactive microsphere counting. The umbilical placental resistance was increased (0.25 to 0.35 mm Hg · ml · min^{-1}) by embolization, and there was an increase in the umbilical artery systolic/diastolic ratio. Embolization produced a significant fall in umbilical flow expressed either as total flow (312 to 237 ml · min^{-1}) or when normalized by reference against splanchnic flow (3.36 to 1.53). We conclude that the umbilical artery flow velocity waveform systolic/diastolic ratio measures the reflection coefficient at the peripheral vascular bed (the "resistance vessels") of the placenta. (Am J Obstet Gynecol 1987;157:1443-8.)

Key words: Umbilical blood flow, flow velocity waveforms, embolization, Doppler ultrasound

Studies of umbilical artery flow velocity waveforms in normal human pregnancy[1] indicate a pattern of high diastolic flow velocity (point B) relative to the systolic peak velocity (point A) (low A/B or peak systolic to least diastolic ratio). With advancing gestational age the ratio of peak systolic to least diastolic velocity (A/B) decreases. This pattern has been assumed to be due to the low resistance of this vascular bed. Umbilical circulation resistance has been directly measured in ovine pregnancy[2,3] and found to decrease with increasing gestational age.

The umbilical artery velocity waveform pattern is quite different in situations of antenatal fetal compromise, as indicated by subsequent birth of a small for gestational age infant. The diastolic flow velocity (B) is low relative to the systolic peak velocity (A), so that the systolic/diastolic (A/B) ratio is high.[1,4,5] A placental microvascular correlative study has demonstrated a loss of small arteries in the tertiary villi of the placentae of human patients exhibiting a high systolic/diastolic (A/B) velocity ratio.[6] The loss of these vessels would be expected to increase resistance, because they are the resistance vessels of the fetal placental circulation.

Our purpose was to examine the relationship between umbilical artery flow velocity waveforms and both volume blood flow and resistance in the umbilical circulation of sheep. Experimental alteration in resistance was produced by a technique of microsphere embolization of the fetal umbilical cotyledonary circulation. Embolization of the ovine maternal uteroplacental circulation with microspheres has been used to alter resistance and decrease flow[7-10] to provide an experimental model of fetal growth retardation. Embolization of the umbilical placental circulation has not been previously reported.

Material and methods

Preparation of animals. Thirteen ewes of known mating date were surgically prepared between days 115 and 120 of gestation. The ewes were fasted for 24 hours before the operation. Anesthesia was induced with 1 gm of thiopental, the ewe was intubated, and anesthesia was maintained by inhalation of a nitrous oxide/oxygen with fluothane mixture. All surgery was done by use of an aseptic technique. The uterus was first exposed by a midline incision, the fetal lamb was palpated, and a small incision was made in the uterus

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Table I. Study results

Parameter	Control			Embolized		
	Day 0	*Day 4*	*Day 9*	*Day 0*	*Day 4*	*Day 9*
Fetal Pa_{O_2} pressure (mm Hg)	20.5 ± 1.1	21.1 ± 1.6	19.1 ± 0.7	21.4 ± 0.7	16.3 ± 0.8*	17.1 ± 0.7*
Fetal arterial pH	7.34 ± 0.2	7.34 ± 0.2	7.33 ± 0.01	7.33 ± 0.01	7.34 ± 0.01	7.35 ± 0.01
Umbilical flow (ml · min^{-1})	352 ± 28	373 ± 46	408 ± 45	312 ± 58	300 ± 42	237 ± 50*
Umbilical flow/kg cotyledon (ml · min^{-1} · kg^{-1})	1.18 ± 0.1	1.25 ± 0.2	1.26 ± 0.1	0.93 ± 0.1	0.92 ± 0.2	0.73 ± 0.2*
Umbilical flow/kg fetus (ml · min^{-1} · kg^{-1})	102 ± 7	108 ± 12	110 ± 12	85 ± 16	81 ± 12	63 ± 14*
Umbilical splanchnic flow ratio	3.14 ± 0.36	2.98 ± 0.24	2.53 ± 0.23	3.36 ± 0.48	2.30 ± 0.31*	1.53 ± 0.33*
Umbilical placental resistance (mm Hg · ml^{-1} · min^{-1})	0.16 ± 0.02	0.17 ± 0.03	0.13 ± 0.02	0.24 ± 0.06	0.23 ± 0.04	0.35 ± 0.11*
Umbilical placental resistance/kg cotyledon (mm Hg · ml^{-1} · min^{-1} · kg^{-1})	49.7 ± 6.5	51.9 ± 10.2	42.2 ± 3.5	76.1 ± 17.1	76.0 ± 12.7	106.2 ± 22.8*
Umbilical arterial systolic/diastolic ratio	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	2.5 ± 0.2*	2.6 ± 0.1*
Maternal						
Total uterine flow (ml · min^{-1})	612 ± 62	722 ± 118	838 ± 118	738 ± 107	605 ± 82	750 ± 106
Uteroplacental flow (ml · min^{-1})	448 ± 55	547 ± 99	927 ± 101	557 ± 93	442 ± 78	554 ± 106
Uteroplacental flow (% CO)	9.1 ± 1.4	9.4 ± 1.4	10.3 ± 1.9	11.1 ± 1.2	9.8 ± 0.9	9.2 ± 1.5
Uteroplacental flow (ml · min^{-1}/kg fetus)	131 ± 15	161 ± 30	184 ± 36	147 ± 22	117 ± 20	146 ± 26

Results shown are mean ± SEM.

*$p < 0.05$ in comparison to control observation.

to allow delivery of a hind limb. Polyvinyl catheters were inserted into the fetal aorta via the femoral artery (catheter inside diameter 1.0 mm, outside diameter 1.5 mm, inserted to 10 cm) and into the fetal inferior vena cava via a leg vein (catheter inside diameter 1.0 mm, outside diameter 2.0 mm, inserted to 20 cm). Intra-amniotic catheters (inside diameter 1.5 mm, outside diameter 2.5 mm) were also inserted and attached to the fetal buttock. Catheter locations were confirmed after the death of the fetus. The fetal membranes and uterine incision were closed in two layers. The fetal catheters were filled with heparinized saline solution (25,000 μ in 500 ml) and plugged. They were exteriorized by a flank incision over which a holding pouch was sewn. A polyethylene catheter (inside diameter 1.0 mm, outside diameter 1.5 mm) was also inserted into the left common carotid artery of the ewe and guided down to the apex of the left ventricle under radiologic visualization. Another polyethylene catheter (inside diameter 1.0 mm, outside diameter 1.5 mm) was inserted into one of the saphenous arteries of the ewe and advanced 15 cm to the femoral artery. Maternal catheters were kept in a neck pouch and hind limb pouch, respectively.

The ewe was given procaine penicillin, and streptomycin, 750 mg each, at the time of surgery and daily for the next 3 days. The fetal lamb was given intramuscular cloxacillin (125 mg) at surgery and intravenous penicillin (150 mg) and streptomycin sulfate (100 mg) daily for the next 3 days. All fetal catheters were flushed with heparinized saline solution daily, while the maternal catheters were flushed every second day.

The animals were maintained in metabolic stalls after surgery. They were allowed water ad libitum and fed 1200 gm of 60% lucerne chaff/40% oats in pellet form daily. All studies were conducted with the ewe standing.

Measurements

Blood flow. Radioactive-labeled 15 μm microspheres (New England Nuclear Company, Boston, Mass.) were used to measure maternal and fetal blood flows.[11] The microspheres were labeled with chromium 51, tin 113, ruthenium 103, strontium 85, niobium 95, and scandium 46. Specific activity ranged from 2.5 to 50 counts per microsphere. The sequence in which these were used on the three occasions and whether to ewe or fetus was randomized. Before injection, the microspheres were mixed by an ultrasonicator. Each fetal flow estimation was carried out with approximately 1×10^6 microspheres injected into the inferior vena cava catheter and flushed over 20 seconds. A blood reference sample was taken from the lower fetal aorta by a withdrawal pump over 1 minute (8 ml) beginning just before the injection and ending 30 seconds after the injection was completed. Maternal flow was estimated by injecting at least 20 million microspheres into the left ventricle and taking a 30 ml reference sample over 1.5 minutes from the femoral artery. The location of the ventricular catheter was confirmed before each study by checking the pressure waveforms.

At the end of the experiment the ewes were killed with an overdose of pentobarbital sodium (600 mg), and tissues of interest were dissected, weighed, homogenized, and placed in preweighed counting vials. The uterus (myometrium and endometrium), fetal membranes, and fetal organs were treated in a similar manner. The cotyledons were counted individually to confirm uniform distribution of microspheres to the cotyledons and the results were totaled.

Reference blood and tissue sample vials were counted in conjunction with standard and background vials in an automated gamma counter, and nuclide separation was performed by computer. Flow was calculated as: (Number of microspheres per gram times reference sample flow rate) divided by Number of microspheres in reference sample. Umbilical flow in the two groups was also normalized by expression as a ratio to the total splanchnic flow. Splanchnic flow was calculated as the total of liver, spleen, pancreas, kidney, stomach, and intestine flows. Because the reference sample for microsphere determination of blood flow was taken from the lower aorta, flow to these organs was accurately measured.

Total absolute resistance in the umbilical bed was calculated as the quotient of mean arterial pressure minus central (inferior vena cava) venous pressure and total flow. Resistance was also calculated per kilogram cotyledonary mass.

Umbilical artery flow velocity waveform recording. A 4 MHz continuous wave Doppler transducer and directional Doppler blood velocimeter was used (Model D10, Medasonics, Mountain View, Calif.). With the ewe standing, contact jelly was spread over the lower abdomen and the transducer searched until the characteristic fetal umbilical artery waveform sound was heard. Fetal and maternal signals were distinguished by checking the heart rate. The umbilical artery signal could be displayed along with the umbilical vein signal with flow in the opposite direction. It could be distinguished audibly and visually from signals of the fetal aorta. This feature was used in recognition. Unlike flow in intra-abdominal great veins, flow in the umbilical vein does not show cardiac pulsations. Umbilical artery waveforms were recorded from at least four sites for each study. The data were recorded and stored on audiotape to permit later measurement after spectrum analysis (Medasonics SP25) display. Recordings of selected study periods were transferred to ultraviolet paper (Model FOR 1002 fiberoptic recorder, Medelec, Woking, England) or Polaroid film for measurement. The peak systolic and least diastolic flow velocities were measured and their ratio calculated by averaging the ratio from each site during the study period.

Miscellaneous. Arterial venous and amniotic pressures

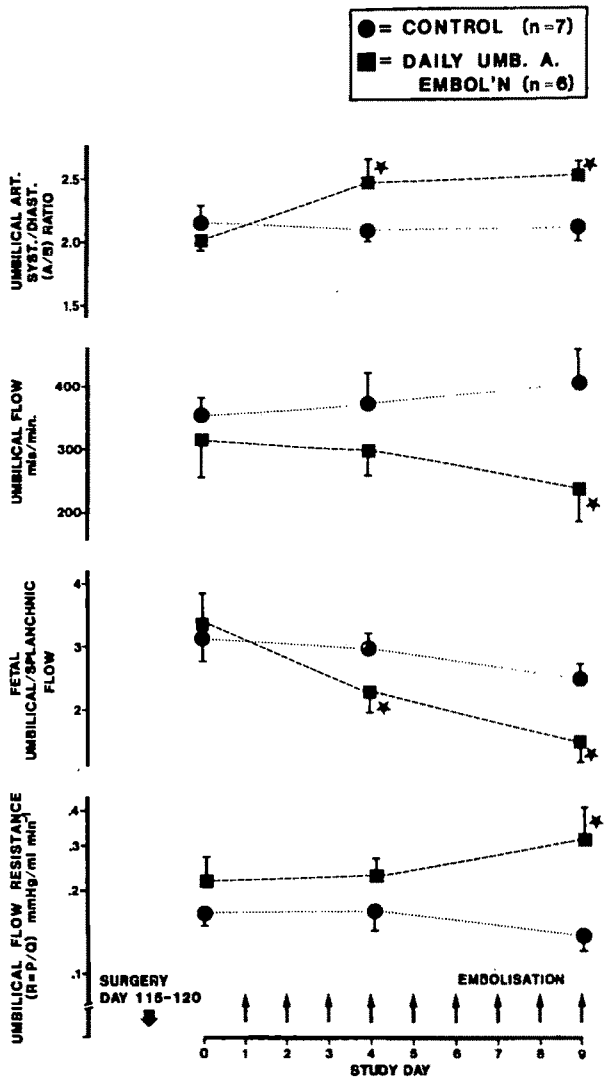


Fig. 1. Mean (±SEM) results of the fetal studies in the control group and in those in whom the umbilical arteries were embolized daily. Significant differences ($p < 0.05$) between the control and embolized groups are shown (*).

were recorded by a pressure transducer (Bell and Howell, Pasadena, Calif.) and a recorder (Model M19, Devices, Cambridge, England). Maternal and fetal heart rates were determined from the arterial pressure waves. Blood gases and pH were measured by a blood gas analyzer (Radiometer, Copenhagen, Denmark).

Experimental protocol. The study began 4 to 7 days after surgery, with seven control and six animals to be embolized. Blood flow with radioactive microspheres was measured at the start of the study and repeated on days 4 and 9 of the experimental period. Blood samples were taken from the ewe and fetus for blood gas analysis just before these measurements. Umbilical artery flow velocity waveforms were recorded daily.

In the umbilical artery embolization group, approx-

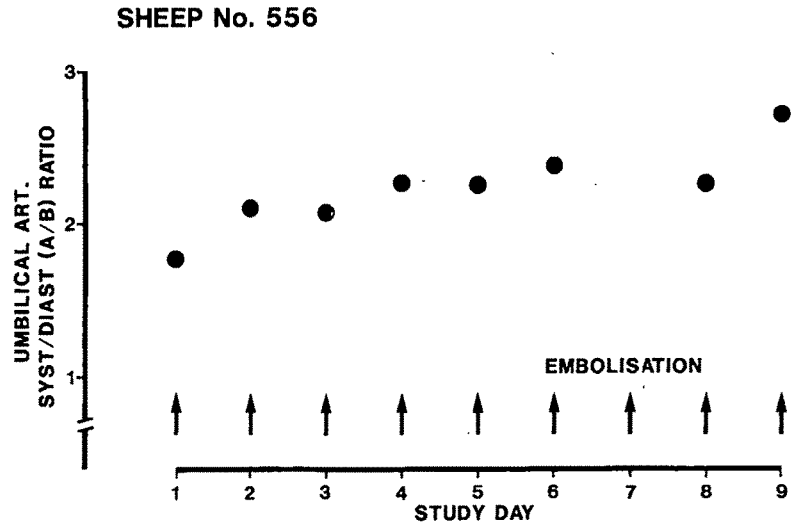


Fig. 2. The umbilical artery systolic/diastolic ratio in one fetal lamb in whom the umbilical circulation was embolized daily.

imately 1.5 million, 15 μm microspheres labeled with cerium 141 were injected each day into the lower abdominal aorta. The use of labeled spheres made it possible to count the number of spheres that traveled via the umbilical arteries to the cotyledons and to check the number of spheres that lodged in the abdominal viscera or lower limbs.

Statistical analysis. Results were analyzed by one-way analysis of variance. Dunnett's test was used for comparison of means between the control and embolized groups and also within the embolized group.[12] A logarithmic transformation was used for the calculated resistance, as this is the ratio of two normally distributed variables and was not normally distributed.

Results

Repetitive embolization of the umbilical placental circulation was associated with an increase in the peak systolic to least diastolic (A/B) ratio of the umbilical artery flow velocity waveform (Table I; Fig. 1). A representative change in this ratio over time in one of the fetuses embolized is shown in Fig. 2. In the control group over the short study period (9 days), there was no significant change in measures of umbilical flow and resistance and the uterine flow (Table I). The control group values did not differ from the baseline (day 0) values in the embolization group. The total umbilical flow was decreased in the embolized group after 9 days ($p < 0.05$) when expressed in absolute terms and relative to fetal weight. When expressed as a multiple of splanchnic flow, this decrease was significant at both 4 and 9 days ($p < 0.05$; Fig. 1). Splanchnic flows did not differ between the groups. Umbilical placental resistance in the embolized group increased and was significant at 9 days ($p < 0.05$). Resistance per unit tissue (cotyledon) mass was also increased at 9 days. Arterial pressure did not vary between the control and embolized groups. Heart rate showed no difference between control and embolized fetuses during the study. On the maternal side of the placenta, total uterine flow and its uteroplacental component were measured (Table I). There was a nonsignificant trend for these to increase in the control and decrease in the embolized group.

Embolization of the umbilical circulation resulted in a fall in oxygen tension in the fetal blood (Table I). Oxygen tension was significantly lower ($p < 0.05$) at both 4 and 9 days. Carbon dioxide tension, pH, and base excess did not differ within or between the two groups.

Of the total dose of embolizing microspheres injected into the lower aorta of the fetus, a mean of 70% was recovered in the cotyledons (range 56% to 78%). There was <3% in the fetal kidneys and <2% in the gut. No cerium 141 microspheres were recovered from the fetal lungs, heart, or brain, indicating that none passed through the placenta. The number of embolizing microspheres in the kidneys was five times the number from a flow measurement. The content of cerium 141 microspheres in the hind limb skin and muscle biopsy specimens was consistent with the approximately 25% lodgement in hind limb. The number of cerium 141 embolizing microspheres in the cotyledons was 16 to 55 (mean 36) times greater than the number of microspheres injected in the course of the three fetal blood flow measurements. The mean fetal weight of the control (3.44 kg) and embolization (3.74 kg) groups

did not differ significantly, and the fetal/cotyledon weight ratio was similar in the two groups.

Comment

Embolization of the umbilical circulation was carried out to obliterate the peripheral arterial tree in the fetal cotyledons and to observe the effect on umbilical placental flow velocity waveforms. These have been proposed as a simple and sensitive index of umbilical circulation and related to fetal welfare.[1-4,5] Umbilical placental resistance was increased by embolization. Embolization resulted in a change in the pattern of the umbilical flow velocity waveform, with an increase in the systolic/diastolic ratio. There was a reduction in the volume flow in the umbilical circulation relative to the control group in both absolute terms and when expressed as a multiple of the splanchnic circulation. There was no change in arterial pressure. This may have occurred had we continued further embolization. The data on visceral blood flow does not suggest a shutdown of vascular beds to improve umbilical flow.

The change in flow velocity waveform observed after embolization corresponds to the change theoretically predicted as a result of increased peripheral or downstream resistance. With each cardiac impulse, pressure and flow waves travel out along the arterial tree. Wave reflection occurs downstream at the site of arterial branches and especially at the level of the "resistance" vessels.[14] The extent of reflection (reflection coefficient) is increased by narrowing of these channels[13] (reduced cross-section area of resistance vessels of the vascular bed). The waveform observed is the sum of the incident and reflected (negative) waves so that a reduced diastolic component would be predicted if reflection is increased. The systolic/diastolic (A/B) ratio was suggested to quantitate this effect. Other indices used for this purpose include the pulsatility index and the resistance index. We have compared these and shown them to be very highly correlated in the umbilical circulation.[14]

The change in the systolic/diastolic ratio was significant at 4 days, but umbilical resistance was not significantly different until 9 days. These results illustrate the difference between calculated resistance (mean pressure difference/mean flow) for steady flow and the flow velocity waveform resistance indices. The latter are more directly determined by the extent of wave reflection at the periphery of the arterial tree rather than resistance. Embolization could be expected to decrease the cross-sectional area of the umbilical placental vascular bed and so increase reflection. Resistance may be defined as the energy lost in forcing blood through the arterial tree.[15] This energy loss includes components due to the pulsatile nature of blood flow (this can be assessed by measuring impedance), the dimensions of the arterial tree and the viscoelastic properties of its walls (distensibility), physical properties of the blood, and the reflected waves. The change in systolic/diastolic ratio preceding the change in calculated vascular resistance can be explained if there is an alteration in any of the other factors affecting resistance opposite to the waveform reflection effect. It is also possible that some of the effect of microsphere embolization may have been hidden by the continued growth and expansion of the umbilical placental bed with gestational age. This was the explanation suggested when the same observations were made about the maternal uterine blood flow after embolization of the maternal uteroplacental bed.[8] Against this explanation is the rise in the flow velocity waveform systolic/diastolic (A/B) ratio we found after embolization. It is also possible that the fetal cotyledon circulation has a greater potential for opening collateral channels than observed in some other tissues in response to microsphere embolization. However, this explanation is not likely because there was a downward alteration in flow in the embolized group.

There was a change in umbilical flow at 9 days that was not significant at 4 days, although the umbilical/splanchnic flow ratio was decreased. Evidence of the preservation of umbilical flow despite an increase in systolic/diastolic ratio in human pregnancy exists.[16] Volume flow in the thoracic aorta and umbilical vein was compared in human fetuses with subsequent birthweight appropriate and small-for-gestational age. The umbilical flow was shown to be 40% of aortic flow in appropriate for gestational age fetuses, but 21% in the small for gestational age group. The umbilical volume flow was of the same magnitude in the two groups, yet the systolic/diastolic ratio was increased in the small for gestational age group. Similar findings were reported in growth-retarded fetal lambs.[11] These observations suggest an increase in cardiac output.

The umbilical artery flow velocity waveforms are similar in normal ovine and human pregnancy. In human pregnancy the two umbilical arteries run from the fetus to the placental disc. In ovine pregnancy the umbilical vessels branch to the many cotyledons. For this reason it is necessary to make multiple observations of the umbilical artery at different sites to derive an averaged systolic/diastolic ratio.

We are not aware of any previous reports of embolization of the umbilical circulation. Embolization of the uterine circulation[7-9] has been used to provide a model of fetal growth failure or asphyxia. Ligation of one umbilical artery has also been studied for a similar reason. Because an alteration in umbilical flow velocity waveform as indicated by an increase in the sys-

tolic/diastolic (A/B) ratio is an early feature of fetal growth retardation, with definite placental pathologic correlation[6] and only sometimes a coexistent alteration in the uterine circulation,[5] umbilical artery embolization may provide a better model for the study of intrauterine growth retardation than does uterine embolization. Our study period (9 days) was too short to affect significantly the fetal weight.

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Continuous estrogen/progestin therapy in menopause

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The incidence of vaginal bleeding, endometrial histology, menopausal symptoms, blood pressure, and serum lipid concentrations were evaluated in 26 women who received either continuous conjugated equine estrogens and medroxyprogesterone acetate (group I, N = 16) or cyclic conjugated equine estrogen + medroxyprogesterone acetate (group II, N = 10) over a 9-month treatment period. At the end of therapy, endometrial biopsy specimens in group I revealed inactive endometrium, whereas one of ten biopsy specimens in group II showed proliferative endometrium. Blood pressure, serum total cholesterol, high-density and low-density lipoprotein cholesterol, and triglyceride levels were not significantly altered from baseline in either group during the 9-month treatment. Vaginal bleeding was virtually eliminated in group I by the end of the study, while 80% of the patients in group II continued to have cyclic menses ($p < 0.001$). This pilot study suggests that continuous conjugated equine estrogen plus medroxyprogesterone acetate therapy appears to be a satisfactory method of postmenopausal hormone replacement, effectively reducing menopausal symptoms without any apparent short-term alterations in serum lipids. It has the added benefit of eliminating cyclic menstrual flow. (Am J Obstet Gynecol 1987;157:1449-53.)

Key words: Menopause, estrogen replacement therapy, progestin therapy

Estrogen replacement is an important part of the preventative health care of postmenopausal women. The utilization of estrogens for the relief of menopausal complaints, such as atrophic vaginitis and vasomotor symptoms,[1] and the prevention of osteoporosis[2] is well established. In addition, the postmenopausal use of estrogens may also reduce the incidence and risk of coronary artery disease.[3, 4] Despite these benefits, many women are reluctant to use cyclic estrogen/progestin replacement therapy because of the problems of vaginal bleeding and recurrence of vasomotor symptoms during the week off of therapy. Some women may choose not to use postmenopausal hormone therapy because of the perceived risk of endometrial cancer, even though the addition of a progestin to conjugated equine estrogen will reduce the risk of endometrial neoplasia.[5, 6] Furthermore, progestin-related side effects such as breast tenderness, headache, nausea, abdominal bloating, and depression, are additional reasons for patient dissatisfaction with cyclic therapy. Therefore, we compared continuous estrogen/progestin therapy to cyclic estrogen/progestin therapy, assessing the relief of vasomotor symptoms and the effect of therapy on blood pressure, endometrial histology, lipid profiles, and the occurrence of uterine bleeding.

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Methods

Menopausal patients with intact pelvic organs who sought estrogen replacement therapy for the relief of menopausal symptoms from the Reproductive Endocrine Division, Department of Obstetrics and Gynecology at the University of South Alabama, were placed in two randomized parallel study groups. Group I consisted of 16 women 42 to 66 years of age, and group II consisted of 10 women 44 to 66 years of age. All patients had been amenorrheic for at least 1 year and had follicle-stimulating hormone (FSH) levels in the menopausal range at the onset of the study. Any patient who was receiving hormone replacement before the start of the study had been amenorrheic for at least 1 year. An elevated FSH level as a baseline, off of estrogen therapy, was a prerequisite to participation in the treatment plan. All patients receiving hormone replacement at the onset of the study had a 6-week washout period before inclusion in either study group. Patients with unexplained vaginal bleeding, liver disease, ischemic heart disease, hyperlipidemia, diabetes, acute or recent vascular thrombosis, or carcinoma of the breast or endometrium were excluded from the study. Informed consent regarding estrogen/progestin replacement therapy and the patient's inclusion in the study protocol was obtained from each patient at the onset of participation in the study. The protocol was approved by the Committee for the Protection of Human Subjects at the University of South Alabama.

The study encompassed a 9-month treatment period in which group I was given 0.625 mg conjugated

Table I. Survey of symptoms

Please rate your symptoms according to the following scale: 0 = no complaint 1 = slight 2 = moderate 3 = marked or severe	
Hot flashes	
Vaginal dryness	
Insomnia	
Depression	
Irritability	
Fatigue	
Lack of sex drive	
Headaches	
Bloating	
Breast tenderness	
Menstrual periods	
Intermittent spotting	
Cramping	
Other	

equine estrogen (Premarin; Ayerst Laboratories, Inc., New York, New York) and 2.5 mg medroxyprogesterone acetate (Provera; Upjohn Manufacturing Company, Kalamazoo, Michigan) daily. Group II received 0.625 mg conjugated equine estrogen daily during days 1 through 25 of each cycle and medroxyprogesterone acetate 10 mg daily during days 16 to 25.

A baseline endometrial biopsy specimen, fasting lipid profile, weight, blood pressure, and a 13-symptom diary (Table I) were obtained from each patient before the start of therapy. All of these factors were reassessed after 3 and 9 months of therapy in group I and at 9 months in group II. Endometrial biopsy specimens were obtained by Novak curette or endometrial pipelle (Unimar, Inc., Willmington, Connecticut) from the uterine fundus. In group II, biopsies were performed during the progestin phase of the cycle. The biopsy specimens were reviewed by a pathologist and one of the physician members of the Division of Reproductive Endocrinology, with concurrence by all interpreters. Fasting lipid profiles obtained at 8:00 AM included cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride levels. Samples from group II were obtained during the later portion of progestin therapy. Repeat lipid profiles were measured after 3 months of therapy in group I, to ascertain if demonstrable adverse effects of combined estrogen-progestin therapy on lipids would warrant discontinuation of treatment. Lipid profiles were obtained at 9 months of therapy in both groups.

The total cholesterol level was measured by the enzymatic method of Cholesterol Rate Dristat No. 6804006 (Beckman Instruments, Somerset, N.J.), and the triglyceride level was measured by the Triglyceride UV Rate Dristat No. 68323 (Beckman Instruments). Both were analyzed by an RA 1000 Discrete Analyzer (Technicon, Raritan, N.J.). HDL cholesterol was measured by a rapid precipitation technique with equal volumes of 2 mol/L $MgCl_2$ and dextran sulfate (molecular weight 500,000; 2 gm/dl) with 0.1 ml of precipitating agent added to 1.0 ml of serum. The solution was mixed, incubated at room temperature for 10 minutes, centrifuged for 20 minutes, and the supernatant assayed for HDL cholesterol. LDL cholesterol was calculated by the Friedwald equation: LDL = Total cholesterol − HDL cholesterol − (triglyceride divided by 5).[7] An approximation of cardiac risk was assessed by the LDL/HDL ratio.[8] The 13-symptom diary maintained by the patient was assessed and compiled by the investigators.

Data were analyzed by the SAS-5 computer program (SAS Instruments, Inc., Cary, North Carolina) and statistical analysis was performed by analysis of variance, the Duncan-Tukey multiple range tests for variables, Fisher's exact test, and paired t test, with significance regarded as a p value < 0.05. All values reported are the mean ± SEM.

Results

As shown in Table II, age, height, weight, and ponderal index were comparable in both groups. Throughout the treatment period, blood pressure was not significantly altered by either method of hormone replacement (Table III).

Improvement in menopausal symptoms was noted in both groups. The occurrence of hot flashes, atrophic vaginitis, and mastodynia was comparable in both groups in regard to incidence and severity. The duration of vaginal bleeding was greater in group II than in group I. The incidence of symptoms (expressed as percent patients with complaints) is shown in Fig. 1.

Vaginal bleeding was virtually eliminated in group I. After 9 months of therapy, 2 patients experienced one episode of spotting. By contrast, eight of 10 patients in group II continued to have cyclic bleeding during the week of no therapy. The difference between group I and group II regarding vaginal bleeding was significant ($p < 0.001$, Fisher's exact test; Fig. 1).

In group I, at the start of therapy, endometrial biopsy revealed proliferative endometrium in five subjects and inactive endometrium in 11. After 9 months of therapy, inactive endometrium was noted in all subjects.

In group II, before the start of therapy, endometrial biopsy revealed inactive endometrium in eight patients, proliferative endometrium in one, and mild hyperplasia in one. The mild hyperplasia was confirmed by diagnostic dilation and curettage. After 9 months of cyclic

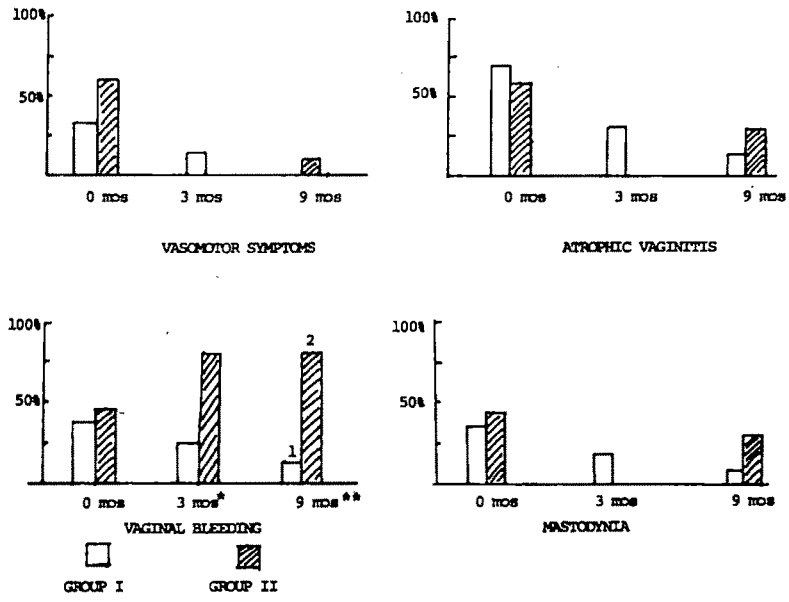


Fig. 1. Percentage of patients with any occurrence of the four listed symptoms without regard to severity. *Open bars* represent group I; *hatched bars* represent group II. Cardiac risk is defined as the LDL/HDL ratio, with an average risk for women of 3.22.[8] *1*, Spotting (one episode); *2*, cyclic bleeding; *p < 0.01; **p < 0.001.

Table II. Mean age, height, weight, and ponderal index at onset of therapy in groups I and II

	Age (yr)	*Height (m)*	*Weight (kg)*	*Ponderal index*
Group I	52.6 ± 1.66	1.65 ± 0.02	62.2 ± 2.43	3.34 ± 0.04
Group II	51.2 ± 2.13	1.62 ± 0.02	67.5 ± 3.18	3.46 ± 0.06

Data are $\bar{x}$ ± SEM.

conjugated equine estrogen + medroxyprogesterone acetate therapy, endometrial biopsy revealed inactive endometrium in nine subjects, while the remaining subject showed a conversion from inactive to proliferative endometrium.

As shown in Table IV, total cholesterol did not change significantly during the course of therapy in either group. In group I, the mean HDL cholesterol concentration at 3 months was comparable to baseline. However, a trend toward an increase in HDL cholesterol levels was noted at 9 months. In group II, a trend toward an increase in HDL cholesterol and a decrease in LDL cholesterol was noted over the 9-month treatment. These changes were not statistically significant.

Comment

We have demonstrated that continuous estrogen/progestin therapy and cyclic estrogen/progestin therapy were equally effective in relieving symptoms of atrophic vaginitis and hot flashes. The use of estrogen replacement therapy did not cause any significant side effects, including a change in blood pressure. This is in agreement with results obtained by other investigators.[9, 10]

Table III. Mean values of blood pressure at onset of study and at 9 months of therapy in groups I and II

Treatment group	*Blood pressure (mm Hg)*	
	Systolic	*Diastolic*
Group I		
0 mo	113.80 ± 4.48	71.00 ± 2.40
9 mo	119.77 ± 3.51	73.77 ± 3.50
Group II		
0 mo	126.57 ± 6.57	78.00 ± 2.61
9 mo	114.25 ± 4.48	68.75 ± 4.19

Data are $\bar{x}$ ± SEM.

It has been suggested that one of the major benefits of estrogen replacement therapy may be the cardioprotective effect of estrogen-induced changes in serum lipids.[11, 12] The increase in HDL cholesterol is thought

Table IV. Mean values of cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, and cardiac risk for groups I and II

	Group I			*Group II*	
	0 mo	*3 mo*	*9 mo*	*0 mo*	*9 mo*
Cholesterol (mg/dl)	231.12 ± 11.49	230.00 ± 11.64	233.58 ± 11.14	227.33 ± 9.46	226.60 ± 15.62
HDL (mg/dl)	54.69 ± 3.47	55.33 ± 3.33	61.67 ± 3.32	54.75 ± 6.56	68.20 ± 7.40
LDL (mg/dl)	140.50 ± 7.97	143.00 ± 9.37	142.67 ± 10.12	147.25 ± 12.70	131.40 ± 18.11
Triglyceride (mg/dl)	140.76 ± 17.51	161.00 ± 20.19	146.00 ± 15.20	154.44 ± 31.76	134.40 ± 31.74
Cardiac risk factor (LDL/HDL ratio)	2.68 ± 0.41	2.72 ± 0.41	2.45 ± 0.39	2.78 ± 0.63	2.24 ± 0.67

Date are $\bar{x}$ ± SEM.

to be the most cardioprotective factor.[13, 14] However, various progestational agents used to counteract the estrogen effect on the endometrium have been reported to reverse this beneficial effect of estrogen.[15, 16]

Although a trend toward increased HDL cholesterol was noted in women receiving estrogen therapy, we were not able to show a definite beneficial effect of estrogen on serum lipids in either group. A temporary beneficial effect on serum lipid concentrations during the estrogen phase of therapy that was reversed during progestin administration is possible. As demonstrated by others,[16, 17] our study with low-dose medroxyprogesterone acetate showed no statistically significant change in HDL cholesterol concentrations over the 9-month treatment. This is comparable to Weinstein's recent data.[18] Whether the reversal of the estrogen-induced changes in lipids by medroxyprogesterone acetate will abrogate the apparent cardioprotective benefit of estrogen therapy is unknown. The trend toward an increase in HDL cholesterol levels in both groups requires additional evaluation and would be best delineated with an extended period of study of larger groups. Our preliminary study would indicate that lipid parameters, HDL cholesterol in particular, are comparable in women receiving cyclic or combined continuous therapy and in women without estrogen/progestin replacement.

Although our short-term combined estrogen/progestin study cannot address the issue of osteoporosis, medroxyprogesterone acetate as well as conjugated equine estrogen have been associated with improvement in the retardation of bone loss. It has been suggested that estrogen and progestin may act synergistically to improve bone metabolism.[19]

Vaginal bleeding was virtually eliminated in the group receiving continuous estrogen/progestin therapy in our study, while cyclic vaginal bleeding was common in the traditional cyclic therapy groups. Our findings are in contrast with those of Clisham et al.,[20] who continuously administered 0.625 or 1.25 mg conjugated equine estrogen and 10 mg medroxyprogesterone acetate but were unable to avoid vaginal bleeding in their patients. The high incidence of vaginal bleeding they noted may have been due to progestational atrophy, as bleeding decreased after the dose of conjugated equine estrogen was increased to 1.25 mg daily. In our study, the incidence of vaginal bleeding at 3 months of therapy in the continuous regimen was significantly less than in the cyclic group ($p < 0.01$, Fisher's exact test), and was less common than that reported by Weinstein[18] (25% versus 50%), who used a dosage schedule comparable to ours for only 3 months. With continued treatment past 3 months, the incidence of vaginal bleeding in our study was almost eliminated.

By the use of low-dose (2.5 mg) medroxyprogesterone acetate daily in our treatment program, we were able to prevent the development of endometrial hyperplasia and avoid vaginal bleeding. The apparent protective effect of low-dose progestin treatment on the endometrium and the absence of vaginal bleeding has been previously reported with the use of norethindrone.[21]

The use of continuous conjugated equine estrogen + medroxyprogesterone acetate therapy for the treatment of menopausal symptoms should gain acceptance by most menopausal women. It effectively controls the symptoms of menopause with the added benefit of eliminating vaginal bleeding. The apparent lack of side effects, the protective effect on the endometrium, and the potential benefit of preventing osteoporosis makes this treatment regimen attractive. Although a beneficial effect on serum lipids could not be demonstrated with this therapy, lipid parameters were not different when no treatment was used. Longer and extended longitudinal studies are required not only to assess potential serum lipid alterations, but also to ascertain the long-term beneficial or adverse effects of this mode of therapy.

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Arachidonate lipoxygenase metabolites in amniotic fluid of women with intra-amniotic infection and preterm labor

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This study was undertaken to examine the effects of intrauterine infection and preterm labor on the amniotic fluid concentrations of arachidonate lipoxygenase metabolites in women with premature rupture of membranes. Amniotic fluid was obtained from four groups of women with premature rupture of membranes: group 1, women without labor or infection; group 2, women with labor but without infection; group 3, women with intra-amniotic infection but without labor; and group 4, women with both infection and labor. 12-Hydroxyeicosatetraenoic acid, 15-hydroxyeicosatetraenoic acid, and leukotriene B_4 were measured by radioimmunoassays. Amniotic fluid concentrations of 12-hydroxyeicosatetraenoic acid were found not to differ significantly among the four groups. Amniotic fluid concentrations of 15-hydroxyeicosatetraenoic acid in group 4 were significantly higher than in women in groups 1 and 3 ($p < 0.05$). In addition, amniotic fluid concentrations in leukotriene B_4 were significantly higher in group 4 than in any of the other three groups ($p < 0.05$). Leukotriene B_4 concentrations were higher in groups 2 and 3 than in group 1, suggesting that the presence of both labor and infection increases the concentration of this metabolite in amniotic fluid. Infection and labor had an additive effect in the elevation of amniotic fluid concentrations of leukotriene B_4. These results suggest that the amniotic fluid concentrations of arachidonate lipoxygenase metabolites are affected differently by the presence of infection and labor in women with premature rupture of membranes. (AM J OBSTET GYNECOL 1987;157:1454-60.)

Key words: Arachidonate lipoxygenase, intra-amniotic infection, premature rupture of membranes

Intra-amniotic infection is frequently associated with the onset of labor.[1] The mechanisms responsible for parturition in these cases have not been established. Parturition at term is associated with mobilization of arachidonic acid from fetal membranes.[2] Arachidonic acid can be metabolized either by way of the cyclooxygenase or lipoxygenase pathways. The former pathway leads to the synthesis of prostaglandins and thromboxanes, whereas the latter pathway leads to the formation of lipoxygenase products such as hydroxyeicosatetraenoic acids and leukotrienes. Arachidonate lipoxygenase products are generated during the course of inflammatory reactions[3] and can be synthesized by human intrauterine tissues.[4] These products have been found to be involved in the development of uterine contractions,[5,6] and they may participate in the normal process of labor or in the mechanism of labor that is associated with or the result of an inflammatory reaction (e.g., chorioamnionitis). The purpose of this study was to determine the concentrations of arachidonate lipoxygenase metabolites in amniotic fluid of women with and without intra-amniotic infections and to evaluate the relative influence of labor and infection on the amniotic fluid levels of these compounds.

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Material and methods

Study design. A cross-sectional study was constructed according to the results of amniotic fluid cultures in women with premature rupture of membranes. Four groups were created: group 1, women without labor or infection; group 2, women with labor but without infection; group 3, women with an intra-amniotic infection but without labor; and group 4, women with both infection and labor. An intra-amniotic infection was defined as the presence of a positive amniotic fluid culture. None of the patients in this study developed

signs and symptoms of infection. Labor was defined as the presence of regular uterine contractions that led to cervical dilatation and spontaneous vaginal delivery within 24 hours of the performance of the amniocentesis. Pelvic examination was not performed at the time of amniocentesis, since this could result in a higher incidence of infection.[7] Women were classified according to the results of amniotic fluid culture and their labor status at the time of amniocentesis.

Retrieval of amniotic fluid. Amniotic fluid was obtained by transabdominal amniocentesis in women admitted to Yale–New Haven Hospital with the diagnosis of rupture of membranes at a gestational age of less than 37 weeks. Our success rate in obtaining fluid from these women is 95%. Amniotic fluid was processed for Gram stain, culture, lecithin/sphingomyelin ratio, and phosphatidylglycerol, and any remaining fluid was stored at −20° C. Immediately before amniocentesis, the presence or absence of uterine contractions was recorded by external tocodynamometry.

Culture technique. Amniotic fluid obtained by transabdominal amniocentesis was transported to the laboratory in a capped, plastic syringe immediately after collection of the specimen. These conditions precluded air contact with the specimen. Plating occurred within 30 minutes of collection in all cases. The samples were plated on blood agar, MacConkey agar, Columbia colistin–nalidixic acid agar, chocolate agar (36° C in 8% carbon dioxide) for aerobic culture and blood agar, bacteroides and bile-esculin agar/laked kanamycin and vancomycin agar, and Martin-Lewis agar (all prereduced) for anaerobic culture. Amniotic fluid was also inoculated in thioglycolate broth. Anaerobic cultures were grown in an anaerobic chamber in a Forma Scientific anaerobic system (Model 1024, Division of Mallinckrodt, Marietta, Ohio). The number of colony-forming units per milliliter was determined by plating the specimen with a 0.01 and 0.001 calibrated urine loop. Each colonial morphotype was identified to species by Food and Drug Administration-Centers for Disease Control–approved commercial systems supplemented by conventional tests when needed. An identification was accepted only when it yielded a normalized probability of 97% or greater. When more than three organisms were grown from an amniotic fluid sample, the infection was referred to as "mixed aerobic or anaerobic flora." Species identification was not conducted in these cases. This protocol evolved, since it was frequently difficult to identify all bacterial species involved, and there was no evidence that this effort altered patient management. There was also no evidence that amniotic fluid concentrations of lipoxygenase products would be preferentially affected by some organisms. Identification of anaerobes was conducted with the IDS RapID ANA system (Innovative Diagnostic System, Atlanta, Ga.). Agar was obtained from Baltimore Biological Laboratories, Cockeysville, Md.

***Mycoplasma* and *Ureaplasma* cultures.** Amniotic fluid was centrifuged at 2000 g for 20 minutes and the sediment was placed into 2 ml of holding media (pleuropneumonia-like organism broth, which was supplemented with fresh yeast extract and horse serum, penicillin, polymyxin B, and amphotericin). Samples were stored at −70° C until culture (Mycotrim-GU, Berkeley, Calif.).

Lipoxygenase metabolite determinations. Arachidonate lipoxygenase products were extracted from amniotic fluid into acidified diethyl ether (five volumes). The solvent was evaporated under nitrogen, and the residue was dissolved in assay buffer and assayed using specific radioimmunoassays for 15-hydroxyeicosatetraenoic acid, 12-hydroxyeicosatetraenoic acid, and leukotriene B_4 (Seragen, Inc., Boston, Mass.). Extraction efficiency was determined by assessment of the recovery of each radiolabeled lipoxygenase product added to an aliquot of an amniotic fluid pool. The extraction efficiency was 76% for leukotriene B_4, 91% for 12-hydroxyeicosatetraenoic acid, and 87% for 15-hydroxyeicosatetraenoic acid. All measurements of amniotic fluid samples were conducted in the same assay. One hundred microliters of amniotic fluid was used in the radioimmunoassay. Intra-assay coefficients of variation were 12% for leukotriene B_4, 11% for 15-hydroxyeicosatetraenoic acid, and 10% for 12-hydroxyeicosatetraenoic acid. Sensitivities of the assays for 12-hydroxyeicosatetraenoic acid and 15-hydroxyeicosatetraenoic acid were 8 pg, whereas it was 4 pg for leukotriene B_4. Inhibition of the binding of the radioligand to the antibody by 50% was achieved by 90 pg for leukotriene B_4, 170 pg for 15-hydroxyeicosatetraenoic acid, and 260 pg for 12-hydroxyeicosatetraenoic acid. Serial sample dilutions were parallel to the standard curve for each assay (slope comparisons: $p > 0.05$). Solvent blanks for leukotriene B_4, 15-hydroxyeicosatetraenoic acid, and 12-hydroxyeicosatetraenoic acid were 8 pg, 29 pg, and 98 pg, respectively. Solvent blanks were subtracted from measured values.

Statistical analysis. The Kolmogorov-Smirnov test was used to test the hypothesis that lipoxygenase products were normally distributed. Logarithmic transformation was required for normality in 15-hydroxyeicosatetraenoic acid. The amniotic fluid concentrations of 12-hydroxyeicosatetraenoic acid were normally distributed, but those of leukotriene B_4 were not. One-way analysis of variance was used to compare the levels of lipoxygenase metabolites in the four different groups for 12-hydroxyeicosatetraenoic acid and log 15-hydroxyeicosatetraenoic acid. Duncan's multiple

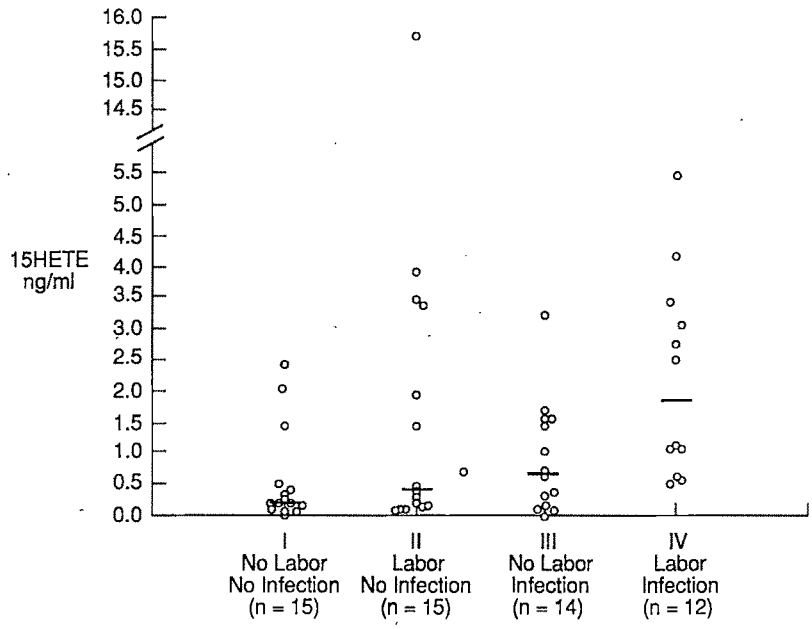


Fig. 1. Amniotic fluid concentrations of 15-hydroxyeicosatetraenoic acid (15-HETE) in the following four groups of women: group 1, women without labor or infection; group 2, women with labor but without infection; group 3, women with an intra-amniotic infection but without labor; and group 4, women with both infection and labor. Logarithmic transformation was required for normality to be achieved. One-way analysis of variance was used to compare the amniotic fluid concentrations of 15-hydroxyeicosatetraenoic acid among the four different groups ($F = 4.02$; $p < 0.05$). A significant difference was found only between groups 1 and 4 and groups 3 and 4 ($p < 0.05$, Duncan's multiple range test). The median concentration of each group is represented by the line in the scattergram of each group.

range test was used for comparison between the different groups. A p value <0.05 was considered statistically significant. Kruskal-Wallis test was used to compare the amniotic fluid concentrations of leukotriene B_4 among different groups. A logarithmic transformation was not used because many patients had nondetectable levels. Multiple comparisons among the four groups were carried out following the procedure described by Conover.[8]

Results

The concentrations of 15-hydroxyeicosatetraenoic acid, leukotriene B_4, and 12-hydroxyeicosatetraenoic acid in amniotic fluid from women in the four different groups are illustrated in Figs. 1 through 3, respectively. The concentration of 12-hydroxyeicosatetraenoic acid in amniotic fluid did not vary significantly among groups (analysis of variance, $p > 0.05$), whereas differences were detected in the levels of 15-hydroxyeicosatetraenoic acid and leukotriene B_4 (analysis of variance, $p < 0.05$). Amniotic fluid concentrations of 15-hydroxyeicosatetraenoic acid were greater in group 4 than in group 1 ($p < 0.05$, Duncan's multiple range test) and greater in group 4 than in group 3 ($p < 0.05$, Duncan's multiple range test). Amniotic fluid concentrations of leukotriene B_4 were significantly different among all groups except for the comparison between groups 2 and 3. Table I illustrates the descriptive statistics for each group. Tables II and III show the results of quantitative microbiology and amniotic fluid concentrations of lipoxygenase products of women with intra-amniotic infections (groups 3 and 4). No consistent relationship between arachidonic lipoxygenase metabolite concentrations in amniotic fluid and specific organisms isolated from the fluid could be demonstrated. No difference in the time interval between amniocentesis and delivery was found in the groups of women in labor at the time of the amniocentesis (groups 2 and 4) ($p > 0.5$, Wilcoxon rank test).

Comment

The results of this study suggest that the amniotic fluid concentrations of 12-hydroxyeicosatetraenoic acid, 15-hydroxyeicosatetraenoic acid, and leukotriene B_4 are affected differently by infection and labor in women with premature rupture of membranes. No differences were detected in the levels of 12-hydroxyeicosatetraenoic acid in amniotic fluid among the four groups; this was not the case with amniotic fluid concentrations of leukotriene B_4 and 15-hydroxyeicosatetraenoic acid. Therefore it appears that differences exist in the activity of the enzymatic path-

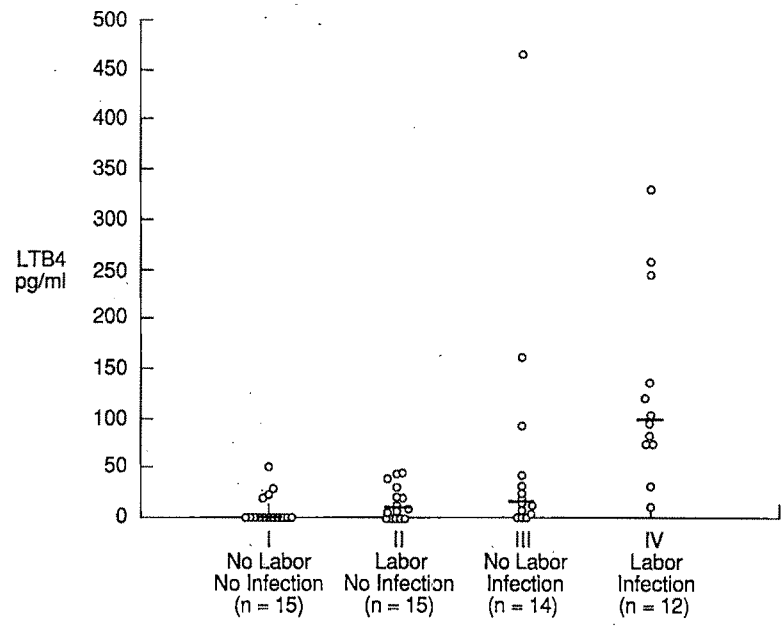


Fig. 2. Amniotic fluid concentrations of leukotriene B_4 (LTB4) in the following four groups of women are displayed: group 1, women without labor or infection; group 2, women with labor but without infection; group 3, women with an intra-amniotic infection but without labor; and group 4, women with both infection and labor. A Kruskal-Wallis analysis of variance was performed to compare amniotic fluid concentrations among the different groups ($p < 0.01$). Amniotic fluid concentrations of leukotriene B_4 were significantly greater in group 4 than in any of the other groups. Amniotic fluid concentrations of leukotriene B_4 were significantly lower in group 1 than in groups 2 and 3. There were no differences between groups 2 and 3 ($p > 0.05$). The median concentration of each group is represented by the line in the scattergram of each group.

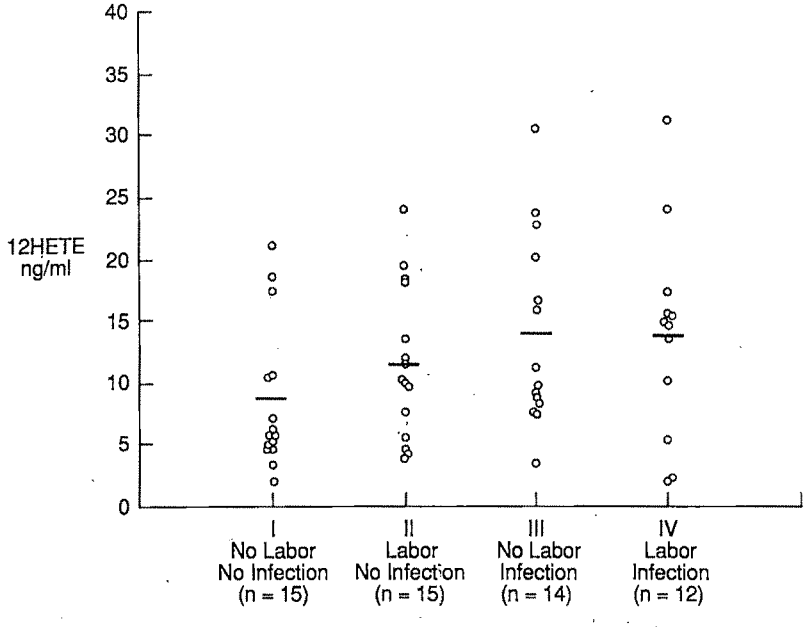


Fig. 3. Amniotic fluid concentrations of 12-hydroxyeicosatetraenoic acid (12-HETE) in the following four groups of women are displayed: group 1, women without labor or infection; group 2, women with labor but without infection; group 3, women with an intra-amniotic infection but without labor; and group 4, women with both infection and labor. One-way analysis of variance was used to compare the amniotic fluid concentrations of 12-hydroxyeicosatetraenoic acid. There were no significant differences in amniotic fluid concentrations of 12-hydroxyeicosatetraenoic acid among the four different groups ($F = 1.80$, $p > 0.05$). The mean concentration of each group is represented by the line in the scattergram of each group.

Table I. Descriptive statistics for concentrations (pg/ml) of amniotic fluid lipoxygenase metabolites in the four study groups

	Group 1	*Group 2*	*Group 3*	*Group 4*
12 HETE				
No. observations	15	15	14	12
Mean	8546	11,496	13,865	14,644
Standard deviation	5873	6182	7792	8432
Standard error of the mean	1516	1597	2082	2434
Median	5773	10,315	10,391	14.644
Range	(2921-21,964)	(4728-24,972)	4296-31,198)	(2910-31,995)
15 HETE				
No. observations	15	15	14	12
Mean	556	2110	908	2192
Standard deviation	761	3994	899	1622
Standard error of the mean	197	1031	240	468
Median	200	386	677	1817
Range	(237-2716)	(382-15,965)	(226-3498)	(788-5744)
LTB4				
No. observations	15	15	14	12
Mean	7.8	15.8	61.6	129.4
Standard deviation	15.0	16.8	124.9	98
Standard error of the mean	3.8	4.3	33.4	28.3
Median	0	10.0	15.5	98.0
Range	(0-50)	(0-46)	(0-465)	(8-331)

Table II. Amniotic fluid concentrations (pg/ml) of arachidonate lipoxygenase products and microbiology of the amniotic fluid in women with intra-amniotic infections but without preterm labor (group III)

Patient identification No.	*Microorganism*	*Colony count**	*12-Hydroxyeicosatetraenoic*	*15-Hydroxyeicosatetraenoic*	*Leukotriene B_4*
111	*Haemophilus influenzae*	$>10^5$	30271	3204	93
141	*Mycoplasma hominis*	$>10^5$	15717	1015	22
195	*M. hominis*	$>10^5$	3369	626	0
	Lactobacillus	$>10^5$			
105	Group B streptococcus	$>10^5$	9131	1440	33
1071	*Fusobacterium* species	—	8121	285	17
	Bacteroides oralis	—			
	M. hominis	—			
36	*Fusobacterium* species	—	8753	107	0
1203A	Group B streptococcus	$>10^5$	11227	348	0
	Fusobacterium species	10^5			
1236A	*M. hominis*	—	22713	728	14
	Ureaplasma urealyticum				
1183A	*Staphylococcus aureus*	$>10^5$	23637	1696	465
1187A	*M. hominis*	$>10^5$	16484	1508	46
1276	*Capnocytophaga*	25×10^5	9555	69	0
1294	*Gardnerella vaginalis*	$>10^5$	20073	0	164
36	*G. vaginalis*	—	7721	141	2
37	*U. urealyticum*	—	7337	1539	7

*Colony count is expressed in colony-forming units per milliliter. When quantitative microbiology was unavailable, it is indicated by —.

ways or the degradation of these compounds in intrauterine tissues. Infection and premature labor may not result in activation of the pathway responsible for the biosynthesis of 12-hydroxyeicosatetraenoic acid; however, these clinical conditions may result in effects on the pathways responsible for the biosynthesis or degradation of 15-hydroxyeicosatetraenoic acid and leukotriene B_4. Alternatively, these results may be explained by differences in the concentration of arachidonic acid. Depending on the Michaelis constant of the enzymes of the different lipoxygenase pathways for arachidonic acid and other substrates, the changes observed could be partially caused by alterations in the concentrations of arachidonic acid.

We have found that premature labor alone in women with premature rupture of membranes does not alter the amniotic fluid concentrations of 12-hydroxyeicosatetraenoic acid or 15-hydroxyeicosatetraenoic acid but

Table III. Amniotic fluid concentrations (pg/ml) of arachidonate lipoxygenase products and microbiology of the amniotic fluid in women with intra-amniotic infections and preterm labor (group IV)

Patient identification No.	*Microorganism*	*Colony count**	*12-Hydroxyeicosatetraenoic*	*15-Hydroxyeicosatetraenoic*	*Leukotriene B_4*
150	*Gardnerella vaginalis*	$>10^5$	14789	5450	83
39	*Fusobacterium* species	$>10^5$	1983	494	246
70	*Mycoplasma hominis*	—	10095	612	134
	Mixed anaerobic flora	3+			
1132	*Fusobacterium* species	$>10^5$	17369	1123	32
33	*G.* vaginalis	1+	24053	2749	72
	Mixed anaerobic flora				
1239A	*Gaffkya anaerobica*	$>10^5$	15402	1075	258
1206A	*G. vaginalis*	$>10^5$	14499	1084	102
1297	*Fusobacterium nucleatum*	25×10^3	2300	549	8
1255	Mixed anaerobic flora	$>10^5$	5255	3060	94
1093	*Listeria monocytogenes*	$>10^5$	13477	2510	72
171A	*Haemophilus influenzae*	$>10^5$	15632	3425	121
1000	*Gardnerella vaginalis*	$>10^5$	31068	4177	331

*Colony count is expressed in colony-forming units per milliliter. When quantitative microbiology was unavailable, it is indicated by —.

only that of leukotriene B_4. This finding is interesting because it appears to be at variance with the results of our earlier study in which women in term labor had significantly elevated concentrations of all these compounds.[9] There are several reasons why such differences may exist. For instance, in our earlier study, all women were in active labor with a cervical dilatation of 6 cm or greater, whereas in the current study, the women are likely to have been in much earlier labor. Data on cervical dilatation are not available because pelvic examinations were not performed in women with premature rupture of membranes, since this procedure may increase the incidence of infection.[7] It can also be argued that a maturational event may account for the differential release of lipoxygenase products into amniotic fluid. Women in preterm labor have lower concentrations of cyclooxygenase metabolites (i.e., prostaglandins) in amniotic fluid and plasma than laboring women at term.[10-12] A similar phenomenon may occur with lipoxygenase metabolites. Evidence supporting this concept is that many women with preterm premature rupture of membranes had nondetectable concentrations of leukotriene B_4 in the present study and that all nonlaboring women at term with intact membranes had detectable concentrations of leukotriene B_4.[9] However, these two groups of women were not identical with respect to membrane status. Intra-amniotic infections alone seemed to affect only the amniotic fluid concentrations of leukotriene B_4 (comparison between groups 1 and 3). It remains to be established whether the increased amniotic fluid concentration of this compound is the result of bacterial or host signals on the metabolism or availability of arachidonic acid.

When both labor and infection are present, the potential relative effects of these two clinical conditions on the amniotic fluid concentration of lipoxygenase metabolites are not easy to interpret. Amniotic fluid concentrations of leukotriene B_4 were significantly elevated in women infected and in labor (group 4) compared with all other groups. This seems to imply that labor and infection have an additive effect on amniotic fluid concentrations of leukotriene B_4. Since amniotic fluid concentrations of prostaglandins are increased with progressive cervical dilatation,[11] it may be hypothesized that the difference observed in amniotic fluid concentrations of leukotriene B_4 between groups 2 and 4 could be attributed to differing cervical dilatation at the time of amniocentesis between the groups. However, this is not likely to be the case because the time interval between amniocentesis and delivery was not different between these two groups.

Significant differences in amniotic fluid concentrations of 15-hydroxyeicosatetraenoic acid were only detected when labor and infection were present (group 4) compared with groups 1 and 3 but not compared with group 2. Further studies will be required to establish the relative contribution of preterm labor and infection to amniotic fluid concentrations of 15-hydroxyeicosatetraenoic acid as this study is not conclusive.

In conclusion, our data indicate that amniotic fluid concentrations of arachidonate lipoxygenase metabolites are affected by the presence of labor and infection in women with preterm premature rupture of membranes. Preterm labor is associated with increased amniotic fluid concentration of leukotriene B_4. Intra-amniotic infection without labor is also associated with increased amniotic fluid concentrations of leukotriene B_4. The presence of preterm labor and intra-

amniotic infection had an additive effect on the increase of amniotic fluid concentrations of leukotriene B_4. Neither preterm labor nor infection was associated with significant changes in amniotic fluid concentrations of 12-hydroxyeicosatetraenoic acid. These observations are consistent with the possibility of an involvement of the lipoxygenase pathway of arachidonic acid metabolism in the mechanism of human parturition associated with preterm labor and intra-amniotic infection.

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Correction

In the article by Key, Giuffrida, and Moore, entitled "Predictive value of early pregnancy glycohemoglobin in the insulin-treated diabetic patient" (Am J Obstet Gynecol 1987;156:1096-100), the abstract is incorrect. The correct abstract appears below.

The influence of early pregnancy glycemic control as measured by hemoglobin A_{1c} concentration and the incidence of congenital anomalies and spontaneous abortions were evaluated in women presenting for prenatal care with insulin-treated diabetes in a population whose glycemic control was poor. Thirty-one abnormal outcomes were seen in 83 pregnancies (37%). There were 22 spontaneous abortions and nine major congenital anomalies. No woman with an early pregnancy hemoglobin A_{1c} value <9.5% had an infant with a congenital anomaly and a single woman experienced a spontaneous abortion (4%). Conversely, in women with an early pregnancy hemoglobin A_{1c} value ≥9.5%, congenital anomalies occurred in 24% and spontaneous abortion in 35%. Outcomes of pregnancies in type I and type II diabetic women were comparable. A strong statistical relationship between hemoglobin A_{1c} and adverse pregnancy outcomes was demonstrated. These results strongly suggest that poor glycemic control during early pregnancy adversely influences pregnancy outcomes; the greater the degree of poor control, the greater the impact on pregnancy outcome. The data further justify the need for preconceptional control in diabetic woman and for careful evaluation of the fetus during pregnancy in the woman with insulin-treated diabetes.

Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor

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This study was undertaken to examine the effects of intrauterine infection and preterm labor on the amniotic fluid concentrations of prostaglandins in women with premature rupture of the membranes. Amniotic fluid was obtained from four groups of patients with premature rupture of the membranes: group 1, patients without labor or infection; group 2, patients with labor but without infection; group 3, patients with an intra-amniotic infection but without labor; group 4, patients with both infection and labor. Prostaglandins E_2 and $F_{2\alpha}$ were measured by radioimmunoassays. Preterm labor, in the absence of infection, was not associated with significant increases in amniotic fluid concentrations of prostaglandins. Women with preterm labor and intra-amniotic infections had higher amniotic fluid concentrations of prostaglandins than women with preterm labor in the absence of infection or women with intra-amniotic infection in the absence of labor. These observations are compatible with the participation of prostaglandins in the mechanisms of onset of preterm labor associated with intra-amniotic infection. (AM J OBSTET GYNECOL 1987;157:1461-7.)

Key words: Prostaglandins, arachidonic acid, premature labor, premature rupture of membranes, intra-amniotic infection, bacteria, labor

Intra-amniotic infection is frequently associated with the onset of labor.[1, 2] The mechanisms responsible for the initiation of parturition under these circumstances have not been established. Several lines of evidence suggest that prostaglandins are involved in the process of normal human parturition.[3-5] It has not been established whether prostaglandins play a role in pathologic human parturition associated with intra-amniotic infection. In a preliminary study we reported that amniotic fluid of women with intra-amniotic infection had higher concentrations of prostaglandins than that of women without infection.[6] However, intra-amniotic infections are frequently associated with the onset of labor. It remains to be established whether this elevation of prostaglandins is secondary to labor, infection, or both. The studies reported in this communication were designed to examine the relationship between intra-amniotic infection and preterm labor and amniotic fluid concentrations of prostaglandins in women with preterm premature rupture of the membranes (PROM).

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Presented in part at the Thirty-third Annual Meeting of the Society for Gynecologic Investigation, Toronto, Ontario, Canada, March 19-22, 1986.

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**Recipient of a Physician Scientist Award from the National Institutes of Health.*

Material and methods

Study design. A cross-sectional study was constructed according to the results of amniotic fluid cultures in women with PROM. Four groups were created: group 1, women without labor or infection; group 2, women with labor but without infection; group 3, women with an intra-amniotic infection but without labor; group 4, women with both infection and labor. An intra-amniotic infection was defined as the presence of a positive amniotic fluid culture whether or not clinical signs of chorioamnionitis were present. Labor was defined as the presence of regular uterine contractions that led to cervical dilatation and spontaneous vaginal delivery within 24 hours of the performance of the amniocentesis. Pelvic examination was not performed at the time of amniocentesis as this could result in a higher incidence of infection.[7] Patients were classified according to the results of amniotic fluid culture and their labor status at the time of amniocentesis. None of the enrolled patients had clinical signs and symptoms of chorioamnionitis.

Retrieval of amniotic fluid. Amniotic fluid was obtained by transabdominal amniocentesis in women admitted to Yale–New Haven Hospital with the diagnosis of PROM at a gestational age of <37 weeks. In our

Table I. Population characteristics of study groups

	Group 1: No labor and no infection	*Group 2: Labor and no infection*	*Group 3: No labor and infection*	*Group 4: Labor and infection*
Age (yr) (mean ± SD)	28.4 + 7.1	24.1 + 5.4	24.5 + 3.5	25.5 + 5.6
Gestational age (wk) (mean ± SD)	32.3 + 3.5*	30.9 + 3.8*	30.0 + 3.5	27.7 + 3.4*
Nulliparous	10	5	5	4
Multiparous	5	10	3	11
Total	15	15	8	15

*Difference between groups 4 and 1, and between groups 4 and 2 are statistically significant (Duncan's range test, $p < 0.05$).

Table II. Organisms isolated from the amniotic fluid in women with intra-amniotic infection (groups 3 and 4)

Organism	*No. of patients*
Streptococcus agalactiae	2
Haemophilus influenzae	2
Viridans streptococci	3
Mycoplasma hominis	5
Mixed anaerobic flora	7
Ureaplasma urealyticum	2
Fusobacterium sp.	6
Gardnerella vaginalis	2
Staphylococci, coagulase-negative sp.	2
Peptococcus sp.	1
Candida albicans	1
Listeria monocytogenes	1
Mixed aerobic flora	1

institution, the success rate in obtaining fluid from these patients is 95%. Amniotic fluid was processed for Gram stain, culture, lecithin/sphingomyelin ratio, and phosphatidylglycerol. Any remaining fluid was stored at −20° C for research purposes. At the time of amniocentesis the presence or absence of uterine contractions was noted.

Culture technique. Amniotic fluid obtained by transabdominal amniocentesis was transported to the laboratory in a capped plastic syringe immediately after collection of the specimen. These conditions precluded air contact with the specimen. Plating occurred within 30 minutes of collection in all cases. The samples were plated on blood agar, MacConkey's agar, Columbia colistin–nalidixic acid agar, chocolate agar (36° C in 8% carbon dioxide) for aerobic culture and blood agar, BBE (*Bacteroides*, bile-esculin agar)/LKV (laked kanamycin, vancomycin agar), and Martin-Lewis agar (all prereduced) for anaerobic culture. Amniotic fluid was also inoculated in thioglycollate broth. Anaerobic cultures were grown in an anaerobic chamber in a Forma Scientific anaerobic system (Model 1024) (Division of Mallinckrodt, Marietta, Ohio). The number of colony-forming units per milliliter was determined by plating the specimen with a 0.01 and a 0.001 calibrated urine loop. Each colonial morphologic type was identified to species by commercial systems approved by the Food and Drug Administration–Centers for Disease Control and supplemented by conventional tests when needed. An identification was accepted only when it yielded a normalized probability of ≥97%. When more than three organisms were grown from an amniotic fluid specimen, the infection was referred to as being due to "mixed aerobic or anaerobic flora." Species identification was not conducted in these cases. This protocol evolved as it is frequently difficult to identify all bacterial species involved.

***Mycoplasma* and *Ureaplasma* cultures.** Amniotic fluid was centrifuged at 2000 g for 20 minutes and the sediment was placed into 2 ml of holding media (pleuropneumonia-like organism broth, supplemented with fresh yeast extract and horse serum, penicillin, polymyxin B, and amphotericin). Samples were stored at −70° C until inoculation into *Mycoplasma* culturing broth took place (Mycotrim-GU, Berkeley, Calif.).[8]

Prostaglandin determinations. Prostaglandins were extracted from amniotic fluid by use of acidified diethyl ether (five volumes). The organic solvent was evaporated under nitrogen and the residue dissolved in phosphate buffer (pH 7.4). Prostaglandin E_2 and prostaglandin $F_{2\alpha}$ were assayed with sensitive and specific radioimmunoassays according to a technique previously described.[9] The intra-assay coefficients of variation were 7% and 12% and the interassay coefficients of variation were 10% and 15%, respectively. The cross-reactivities of antisera with other major prostaglandins were <1%.

Statistical analysis. A Wilk-Shapiro test was used to test the hypothesis that amniotic fluid prostaglandin concentrations in each group of women were distributed normally. A logarithmic transformation was required for the data to achieve normality. One-way analysis of variance was used to compare the amniotic fluid concentrations of prostaglandins in the four different groups. Duncan's tests were used for comparison between the different groups.

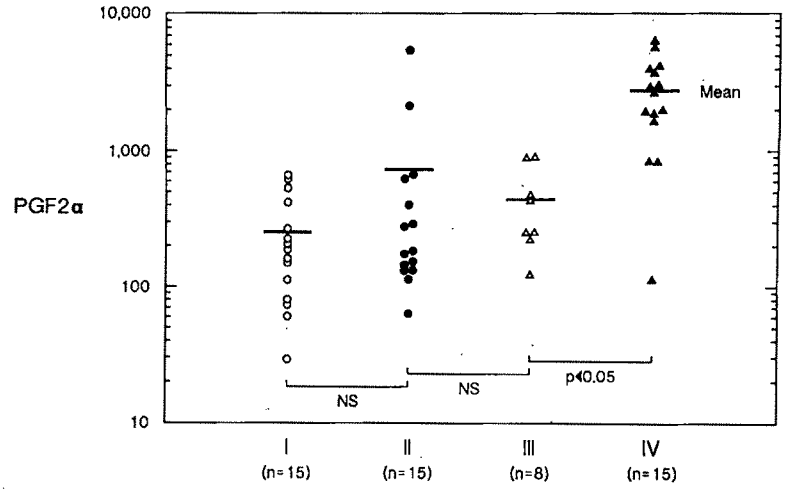


Fig. 1. Amniotic fluid concentrations of prostaglandin $F_{2\alpha}$ in the study groups. Group 1: Women without labor or infection; group 2: women with labor but without infection; group 3: women with intra-amniotic infection but without labor; group 4: women with both infection and labor. Logarithmic transformation was required for normality to be achieved. One-way analysis of variance was used to compare the amniotic fluid concentrations of prostaglandin $F_{2\alpha}$ ($F = 13.13$, $p < 0.001$). Significant differences were found between groups 4 and 3, 4 and 2, and 4 and 1 ($p < 0.05$, Duncan's test).

Table III. Amniotic fluid concentrations (in picograms per milliliter) of prostaglandin $F_{2\alpha}$ in the study groups

	Group 1	*Group 2*	*Group 3*	*Group 4*
No. of observations	15	15	8	15
Mean	252	731	441	2783
SEM	53	364	106	458
SD	207	1409	300	1774
Median	187	183	340	2649
Range	29-61	63-5470	122-900	112-6389

Group 1: Women without labor or infection; group 2: women with labor but without infection; group 3: women with intra-amniotic infection but without labor; group 4: women with both infection and labor.

Table IV. Amniotic fluid concentrations (in picograms per milliliter) of prostaglandin E_2 in the study groups

	Group 1	*Group 2*	*Group 3*	*Group 4*
No. of observations	15	15	8	15
Mean	489	399	722	3047
SEM	120	102	221	554
SD	467	395	626	2145
Median	278	290	502	3143
Range	78-1802	94-1636	119-1921	127-7618

Group 1: Women without labor or infection; group 2: women with labor but without infection; group 3: women with intra-amniotic infection but without labor; group 4: women with both infection and labor.

Results

Groups 1, 2, and 4 consisted of 15 women each. Group 3 (infection and no labor) was composed of eight women. The maternal age, gestational age at the time of amniocentesis, and parity are depicted in Table I. There were no differences in maternal age among women in all groups. Women in group 4 had a significantly lower gestational age than women in groups 1 and 2 ($p < 0.05$, with analysis of variance and Duncan's test).

The organisms isolated from the amniotic fluid of women with intra-amniotic infections (groups 3 and 4) are depicted in Table II. Mixed aerobic infections and mycoplasma species were the most frequent isolates. There was a single case of *Candida albicans* intra-amniotic infection. The two cases of intra-amniotic infections with coagulase-negative staphylococci occurred in patients who had repeated amniocenteses and pathologic evidence of chorioamnionitis on placental examination.

The amniotic fluid concentrations of prostaglandin $F_{2\alpha}$ in the four different groups of women are shown in Fig. 1. Fig. 2 displays amniotic fluid concentrations of prostaglandin E_2. Descriptive statistics for these data are presented in Tables III and IV. The differences between group 4 and all other groups for both prostaglandins E_2 and $F_{2\alpha}$ were significant ($p < 0.05$ in all instances), while the differences among groups 1, 2, and 3 were not significant ($p > 0.05$).

When amniotic fluid concentrations of prostaglandins of women in labor (groups 2 and 4) and women not in labor (groups 1 and 3) are compared, disregarding the results of bacteriologic examination of am-

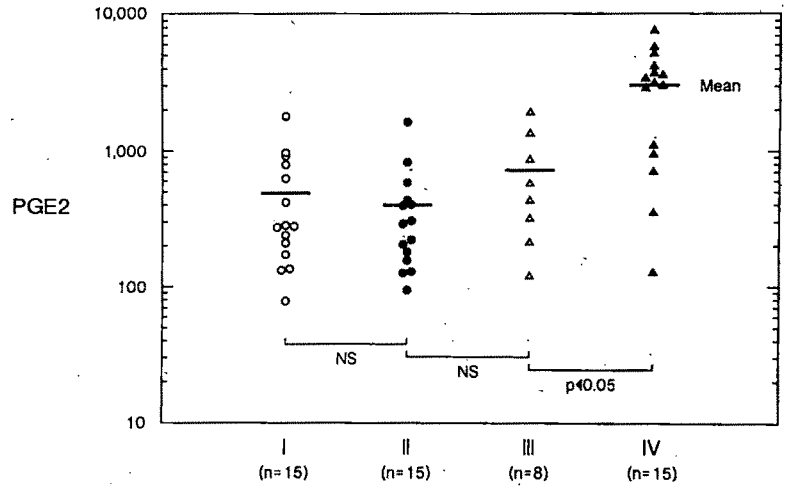


Fig. 2. Amniotic fluid concentrations of prostaglandin E_2 in the study groups. Group 1: Women without labor or infection; group 2: women with labor but without infection; group 3: women with intra-amniotic infection but without labor; group 4: women with both infection and labor. Logarithmic transformation was required for normality to be achieved. One-way analysis of variance was used to compare the amniotic fluid concentrations of prostaglandin E_2 ($F = 18.44$, $p < 0.001$). Significant differences were found between groups 4 and 3, 4 and 2, and 4 and 1 ($p < 0.05$, Duncan's test).

Table V. Microbiologic features and amniotic fluid concentrations of prostaglandin in women in group 3

Case No.	*Organism isolated*	*Colony count*	*Prostaglandin E_2 (pg/ml)*	*Prostaglandin $F_{2\alpha}$ (pg/ml)*
1	*Haemophilus influenzae*	10^5	573	884
2	*Streptococcus agalactiae*	10^5	211	251
3	*Streptococcus agalactiae*	10^5	317	221
4	Viridans streptococci	10^5	119	122
5	*Mycoplasma* sp.	10^1		
	Mixed anaerobic flora		862	474
	Ureaplasma urealyticum			
6	*Mycoplasma* sp.	10^5	1921	900
7	*Fusobacterium* sp.		1345	428
8	*Fusobacterium* sp.			
	Mycoplasma sp.		431	250

niotic fluid, higher concentrations of both prostaglandins could be demonstrated in the laboring group. The amniotic fluid concentrations of prostaglandin E_2 in laboring and nonlaboring women were 1723 ± 370.1 and 570 ± 109.6 pg/ml (mean ± SEM), respectively ($p < 0.05$). The amniotic fluid concentrations of prostaglandin $F_{2\alpha}$ in laboring and nonlaboring women were 1757 ± 344.8 and 318 ± 53 pg/ml, respectively ($p < 0.001$). Similarly, when infected women (groups 3 and 4) are compared with uninfected women (groups 1 and 2), higher amniotic fluid concentrations of prostaglandins are demonstrated in the infected group. The amniotic fluid concentrations of prostaglandin E_2 in infected women and uninfected women were 2238 ± 434 and 444 ± 78 pg/ml (mean ± SEM), respectively (t test, $p < 0.0001$). The amniotic fluid concentrations of prostaglandin $F_{2\alpha}$ in infected and uninfected women were 1968 ± 380 and 491 ± 186 pg/ml, respectively (t test, $p < 0.00001$). Caution is advised in the interpretation of the comparisons between laboring and nonlaboring women and between infected and noninfected women. The design of this study limits extrapolation of these results to a population of women in preterm labor or with intra-amniotic infections without taking into account the interaction of the other variable (labor or infection).

Tables V and VI illustrate the qualitative and quantitative microbiologic features and amniotic fluid-prostaglandin concentrations in infected women of groups 3 and 4, respectively. No appreciable difference was noted in the microbiologic characteristics of the two groups.

Comment

Although increased production of prostaglandins by intrauterine tissues is thought to be a key event in the

Table VI. Quantitative microbiologic characteristics and amniotic fluid concentrations of prostaglandins in women with intra-amniotic infections and preterm labor

Case No.	*Organism isolated*	*Colony count*	*Prostaglandin E_2 (pg/ml)*	*Prostaglandin $F_{2\alpha}$ (pg/ml)*
1	*Gardnerella vaginalis*	10^5	127	3032
	Mixed anaerobic flora	10^5		
2	Staphylococci, coagulase-negative sp.		350	2931
	Peptococcus sp.			
3	*Gardnerella vaginalis*	10^5	943	1646
	Fusobacterium sp.	10^5		
4	*Mycoplasma* sp.	10^5	1101	1933
	Mixed anaerobic flora	10^5		
5	*Fusobacterium* sp.	10^5	3718	2649
6	Mixed anaerobic flora	10^5		
	Staphylococci, coagulase-negative sp.	10^5	3411	112
	Viridans streptococci	10^5		
	Ureaplasma urealyticum			
7	Mixed anaerobic flora	3^+	706	828
	Mycoplasma sp.			
8	Mixed gram-negative aerobic organisms	–	5772	3709
9	Mixed gram-positive and gram-negative anaerobic organisms	–	5147	6389
10	*Fusobacterium* sp.	10^5	2883	1864
11	*Haemophilus influenzae*	10^5	4180	4160
12	Viridans streptococci	10^3	7618	3960
	Mixed anaerobic flora	10^5		
13	*Candida albicans*	2×10^3	3010	832
14	*Listeria monocytogenes*	10^5	3143	1989
15	*Fusobacterium* sp.	10^5	3590	5705

initiation of parturition in human labor at term,[3-5] there is conflicting evidence concerning whether prostaglandins have an equally important role in the onset of preterm labor. Concentrations of prostaglandins in amniotic fluid are increased later in women in preterm labor compared with women in labor at term.[10] Moreover, plasma prostaglandin concentrations in women in preterm labor have been shown not to increase to the same extent as in women in term labor.[11] However, one group has recently reported elevated maternal plasma concentrations of the prostaglandin F metabolite in women with preterm labor refractory to tocolytic therapy.[12] As prostaglandins are inflammatory mediators and bacteria involved in intra-amniotic infection are a potential source of phospholipase A_2 activity,[13] it would seem logical to expect that prostaglandins may play a role in the onset of human labor associated with intra-amniotic infections.

We have been unable to document an increase in the amniotic fluid concentrations of prostaglandins between noninfected women with PROM but without preterm labor and noninfected women with PROM but with preterm labor (groups 1 and 2). There are several possible explanations for these findings. First, the women in group 2 (labor without infection) were in very early labor. Amniocentesis was performed in these women for clinical management as this procedure would not be justified ethically in patients in advanced labor or in those in whom delivery was imminent. It is known that concentrations of prostaglandins in amniotic fluid and plasma rise with progressive cervical dilatation, and therefore the magnitude of the difference in amniotic fluid concentration of prostaglandins is partially dependent on the stage of labor in which the sample of amniotic fluid is obtained. It is possible that women in group 2 may not have reached a point in labor at which a significant difference in amniotic fluid prostaglandin concentrations could have been detected. Second, it is possible that the "control" group (group 1, women without infection or labor) had an increased baseline amniotic fluid concentration of prostaglandins associated with the mechanical trauma of rupture of the membranes. This elevation may obscure the detection of differences between groups 1 and 2. Supporting this view is the observation that amniotomy causes a rapid rise in the amniotic fluid and plasma concentrations of prostaglandin.[14, 15] Third, the failure of amniotic fluid prostaglandin concentrations to be elevated in cases of preterm labor unassociated with intra-amniotic infection may reflect an alteration in the normal signal(s) responsible for the onset of labor. Several lines of evidence suggest that at term such a signal has its origin within the amniotic cavity under normal circumstances. Fetal urine has been proposed to contain a factor capable of inducing the release of prostaglandins from amniotic cells and being such a signal.[17] This would explain the increased levels of prostaglandins in normal term labor. However, if the onset of preterm labor is triggered by bacterial products or inflammatory mediators present in the human decidua and not

in the amniotic cavity, prostaglandin levels in the amniotic fluid may not reach levels comparable to those achieved when the signal is of intra-amniotic origin.

The amniotic fluid concentrations of prostaglandins were not higher in nonlaboring women with PROM and intra-amniotic infection (group 3) than in women with PROM without intra-amniotic infections regardless of the presence or absence of labor (groups 2 and 1). At first, this finding would seem to argue against a role for prostaglandins in the onset of parturition associated with intra-amniotic infection since one would expect higher amniotic fluid concentrations in the infected group. However, this finding may simply reflect a temporal phenomenon. The production of prostaglandins by intrauterine tissues in response to bacterial infection may require a period of time for the generation of sufficient amounts of bacterial products or host inflammatory mediators to induce increased prostaglandin production. This hypothesis is supported by the difference observed between groups 3 and 4. Women in preterm labor with infection had higher amniotic fluid concentrations of prostaglandins than women with infection alone (group 4 versus group 3). These differences cannot be attributed to the presence of preterm labor alone as the amniotic fluid concentrations of prostaglandins were greater in women with preterm labor and infection (group 4) than in women with preterm labor without infection (group 2). This difference is unlikely to be attributable to the stage of labor at which the amniocentesis was performed because no differences in the amniocentesis-to-delivery interval were observed between groups 2 and 4. Although the mean gestational age in group 4 was significantly lower than that of group 2, it is unlikely that this accounted for the results observed because the earlier the gestational age the less likely there would be differences in amniotic fluid or plasma prostaglandin concentrations in women with preterm labor.[10, 11] Therefore the bias in the study is against the observed results. The difference in amniotic fluid prostaglandin concentration between groups 3 and 4 cannot be attributed to variation in quantitative or qualitative microbiology, as these were comparable (Tables III and IV). Therefore the data presented in this communication indicate that amniotic fluid concentrations of prostaglandins are elevated in preterm labor associated with intra-amniotic infection.

The mechanisms responsible for the increased concentration of prostaglandins in laboring women with intra-amniotic infections have not been established. The signal for the increased production of prostaglandins may be of bacterial origin. Lamont et al.[18] have found that bacterial products are capable of increasing the release of prostaglandin E_2 from amniotic cells. An alternative or complementary signal for the initiation of labor in the presence of infection may result from the activity of inflammatory mediators produced by the host in response to bacterial invasion. Interleukin 1, a fever and inflammatory mediator produced by mononuclear cells,[19, 20] could induce prostaglandin biosynthesis by intrauterine tissues. The ability of this hormone to stimulate the production of prostaglandins by other cell types has been demonstrated.[21-23]

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Insulin resistance and abnormal ovarian responses to human chorionic gonadotropin in chronically anovulatory women

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We studied the interrelationships between insulin resistance, obesity, and abnormal ovarian androgen secretion in chronically anovulatory women with clinical or biochemical evidence of hyperandrogenism. Four groups of six subjects each were studied: (1) normal weight (within 10% ideal body weight) anovulatory, (2) obese (>120% ideal body weight) anovulatory, (3) normal weight eumenorrheic, and (4) obese eumenorrheic. After dexamethasone suppression, human chorionic gonadotropin (2000 IU/1.5m^2 body surface area intramuscularly) was administered to each subject. Serum testosterone levels were subsequently determined hourly for 17 hours. On a separate occasion, an oral glucose tolerance test was administered to five subjects from each group. Serum glucose and immunoreactive insulin levels were determined before and after the ingestion of a standard 100 gm glucose load. As a group, the anovulatory women had higher ($p < 0.05$) basal testosterone levels (1005 ± 97 pg/ml) than did the ovulatory women (241 ± 21 pg/ml) (values ± SE). Obesity per se was not associated with increased basal testosterone levels. Testosterone levels rose in response to human chorionic gonadotropin ($p < 0.005$) only in obese anovulatory women, reached maximal levels after 3 hours, and subsequently remained stable. Basal immunoreactive insulin levels were elevated ($p < 0.05$) only in obese anovulatory women (52.4 ± 20 μU/ml) compared with obese eumenorrheic (8.7 ± 1.0 μU/ml), normal weight anovulatory (5.8 ± 2.4 μU/ml), and normal weight eumenorrheic (4.6 ± 0.4 μU/ml) women. Similarly, maximal increases in immunoreactive insulin levels after glucose ingestion were significantly greater ($p < 0.01$) in obese anovulatory women compared with other groups. Of note is the observation that maximal changes in testosterone observed within the first 3 hours after human chorionic gonadotropin and maximal changes in insulin were correlated ($r = 0.91$, $p < 0.01$). These data suggest that (1) both insulin resistance and an abnormal acute response to human chorionic gonadotropin are seen only in obese anovulatory women and (2) the degree to which these two abnormalities are manifested is clearly correlated. The mechanism(s) responsible for this interrelationship, as well as the underlying cause(s) of these biochemical defects, remain to be elucidated. (Am J Obstet Gynecol 1987;157:1468-73.)

Key words: Insulin resistance, abnormal ovarian responses, chronic anovulation

Several groups have demonstrated that insulin resistance in women with polycystic ovary syndrome correlates with the degree of hyperandrogenism.[1-3] This finding was originally described in obese subjects, but more recently has been documented in normal weight subjects who otherwise fulfilled strict criteria for the diagnosis of polycystic ovary syndrome, including perimenarcheal onset of oligomenorrhea, hirsutism, and an elevated ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH).[3] Insulin resistance in women with polycystic ovary syndrome persists despite long-term ovarian suppression, suggesting that the insulin resistance and the resultant hyperinsulinemia may be responsible for the excess ovarian androgen secretion rather than vice versa.[4] This view has been substantiated further by in vitro studies, which have demonstrated the ability of insulin and related growth factors to stimulate androgen production in human ovarian stroma and theca.[5-21] It has been postulated that a local intraovarian hyperandrogenic environment may inhibit ovarian folliculogenesis.[6] This study was designed to define further the interrelationships among chronic anovulation, body weight, insulin resistance, and LH-dependent hyperandrogenism.

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Subjects

We studied 12 women between the ages of 18 and 38 years (mean 25.6 years) with histories of oligomen-

Table I. Characteristics of the subjects and basal hormonal profiles

	Eumenorrheic		*Amenorrheic*	
	Normal weight (n = 6)	*Obese (n = 6)*	*Normal weight (n = 6)*	*Obese (n = 6)*
Age (yr)	25 ± 1.1*	31 ± 2.1	24 ± 1.5	30 ± 1.6
Height (cm)	168 ± 2.8	165 ± 2.1	162 ± 2.4	160 ± 3.0
Weight (kg)	57 ± 2.1	87 ± 7.5	58 ± 2.7	97 ± 5.4
% ideal body weight	102 ± 2.0	163 ± 13	108 ± 2.0	210 ± 21.0
No. with LH/FSH > 2	0	0	1	2
No. with hirsutism	0	0	3	3
No. with perimenarcheal onset of amenorrhea	0	0	4	4
LH (mIU/ml)	8.0 ± 1.0	12.0 ± 2.4	12.0 ± 2.0	15.0 ± 2.2
FSH (mIU/ml)	9.0 ± 0.4	9.0 ± 0.9	10.0 ± 1.1	11.0 ± 2.3
Prolactin (ng/ml)	9.0 ± 0.4	7.0 ± 0.5	10.0 ± 1.9	7.0 ± 1.1
Testosterone (pg/ml)	257 ± 24	224 ± 33.0	873 ± 127	1138 ± 123.0
	241 ± 21		1005 ± 96†	
Insulin (μU/ml)	4.6 ± 0.4	8.7 ± 0.8	5.8 ± 2.4	52.4‡ ± 19.9

*Mean ± SEM.
†$p < 0.05$ versus all menstruating.
‡$p < 0.05$ versus other groups.

orrhea (<four periods/yr) who had clinical or biochemical evidence of hyperandrogenism. All subjects had been amenorrheic for at least 3 months before study and had not ingested any hormonal preparations during this time. Subjects with hypothalamic amenorrhea, hyperprolactinemia, thyroid disease, or primary adrenal disease were excluded. The subjects were divided into two groups on the basis of body weight. Obese amenorrheic subjects exceeded their ideal body weight as determined from the Metropolitan Life Insurance Company tables by 20%.[7] Normal weight amenorrheic subjects were within 10% of ideal body weight.

Twelve age-matched control subjects with regular menstrual periods (cycle length from 26 to 30 days, mean 28 days) were studied during the early follicular phase. Six subjects were obese and six were of normal weight. Basal LH, FSH, and prolactin levels were determined in all subjects before entry into the study. All subjects provided written informed consent before entry into the study.

Methods

Oral glucose tolerance test. All subjects were instructed to eat a 300 gm carbohydrate diet for 3 days before testing and were deprived of food after 6:00 PM the evening before testing. They were subsequently admitted to the Northwestern University Clinical Research Center at 7:00 AM, and an intravenous infusion of normal saline solution was started in a forearm vein.

Three blood samples were drawn at 10-minute intervals before the administration of a 100 gm oral glucose load at 8:00 AM. Subsequent blood samples were obtained 15, 30, 60, 120, and 180 minutes after glucose administration. Glucose and immunoreactive insulin levels were measured in all samples. Glucose concentrations were determined by an automated glucose oxidation method (Beckman autoanalyzer, Beckman Instruments, Palo Alto, California). Basal levels of glucose and insulin were calculated by averaging the values from the first three samples. Five subjects in each of the four study groups were studied in this manner.

Human chorionic gonadotropin (hCG) stimulation test. On a separate occasion, each subject received 2.0 mg of dexamethasone orally between 11:00 PM and midnight on the night before testing and 0.5 mg by mouth at noon, 6:00 PM, and midnight on the day of testing. After admission to the Clinical Research Center and insertion of an intravenous line kept open with normal saline solution, hCG (2000 IU/1.5m^2 body surface area) was administered intramuscularly at 1:00 PM. Blood samples were obtained every hour from 1:00 PM until 6:00 AM the next morning. Testosterone levels were determined in each sample by radioimmunoassay (RIA). All 24 subjects participated in this experiment.

Hormone assays. Circulating levels of immunoreactive insulin were measured by RIA as previously described.[8] Testosterone levels were also measured by RIA (Testosterone In Vitro Diagnostic Kit, Leeco Diagnostic, Inc., Southfield, Michigan). FSH, LH, and prolactin were measured by RIA as previously described.[9-11] The interassay coefficients of variation for the assays were as follows: insulin (10%), testosterone (6.5%), FSH (2.5%), LH (11.9%), and prolactin (8.9%). The intraassay coefficients of variation for the assays were as follows: insulin (5%), testosterone (4%), FSH (3%), LH (3%), and prolactin (3%).

Statistical analysis. All statistical analyses were carried out by means of one- or two-way analysis of var-

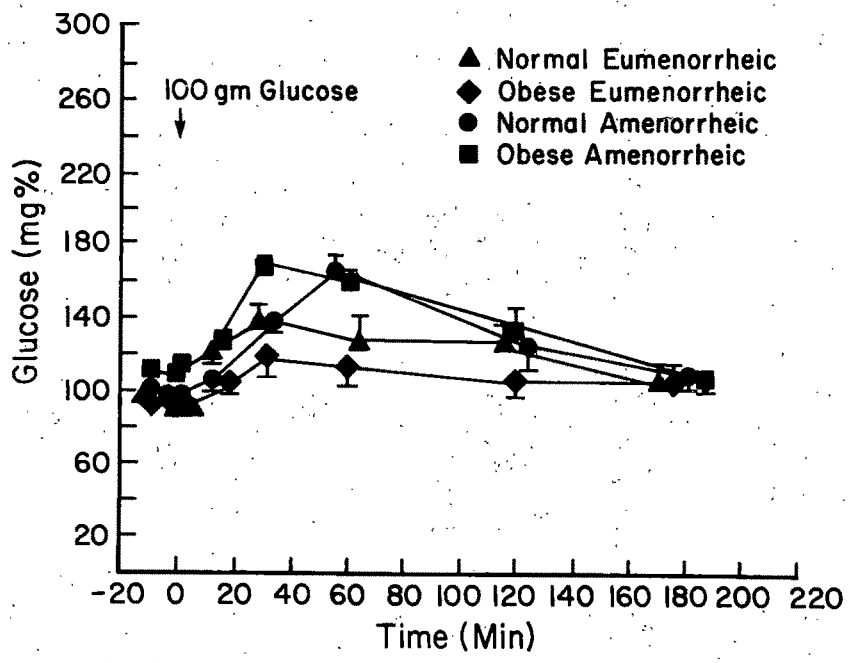


Fig. 1. Changes in serum glucose concentrations in response to a 100 gm oral glucose load in eumenorrheic and amenorrheic women categorized by weight.

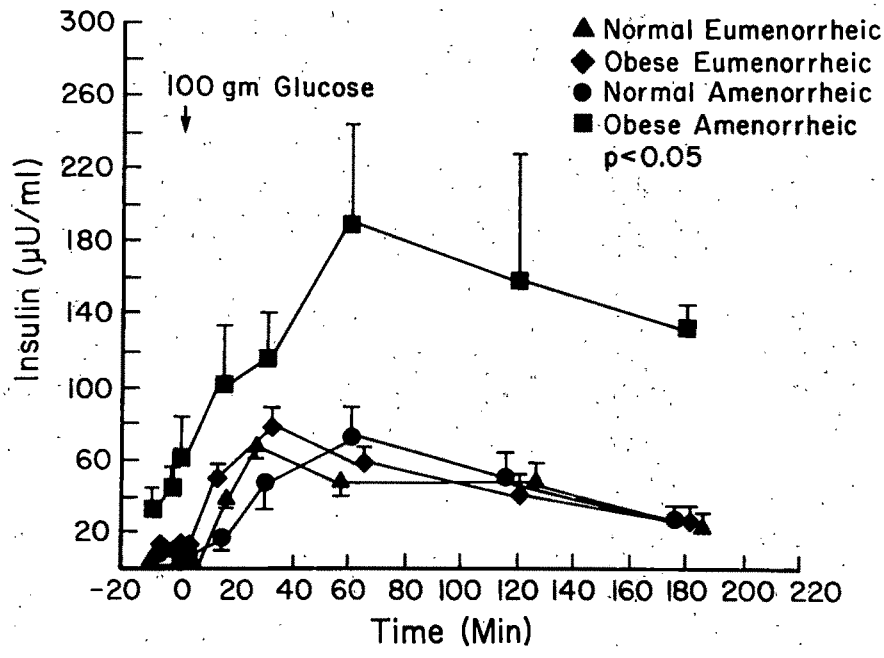


Fig. 2. Changes in serum insulin levels in response to a 100 gm oral glucose load in eumenorrheic and amenorrheic women categorized by weight.

iance with subsequent Duncan's multiple range testing where appropriate. Data are expressed as means ± standard error of the mean. Linear regression analyses were carried out with the method of least squares.

Results

Collectively, the anovulatory subjects had a higher ($p < 0.05$) mean basal testosterone level compared with the eumenorrheic women (Table I). Individually, obese anovulatory women tended to have higher testosterone levels than normal weight anovulatory women, but the differences between the groups were not significant.

Basal glucose levels and those after glucose ingestion were similar in all four groups (Fig. 1). Basal immunoreactive insulin levels were increased ($p < 0.05$) only in obese anovulatory women when the mean values of

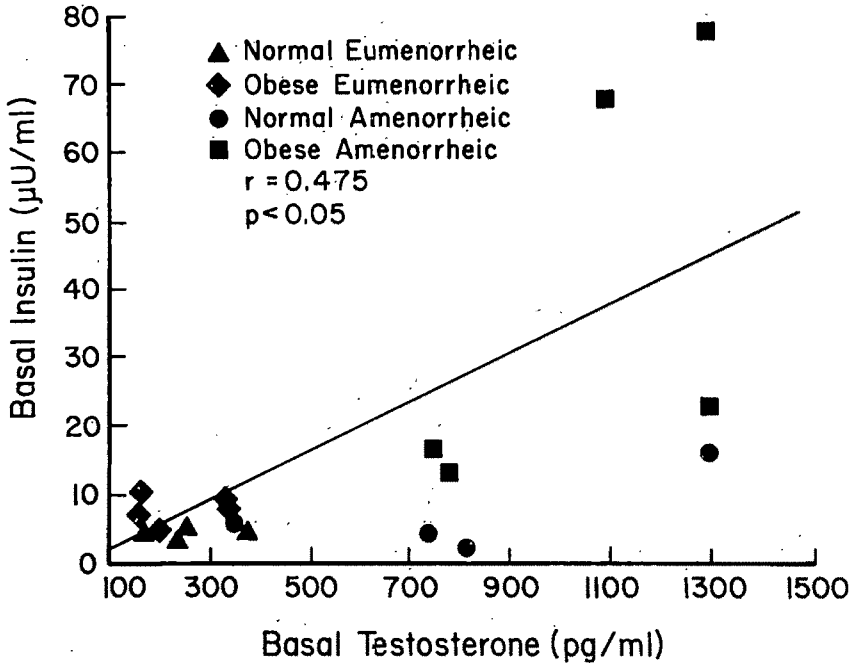


Fig. 3. Relationship between basal testosterone and basal insulin levels in eumenorrheic and amenorrheic women categorized by weight.

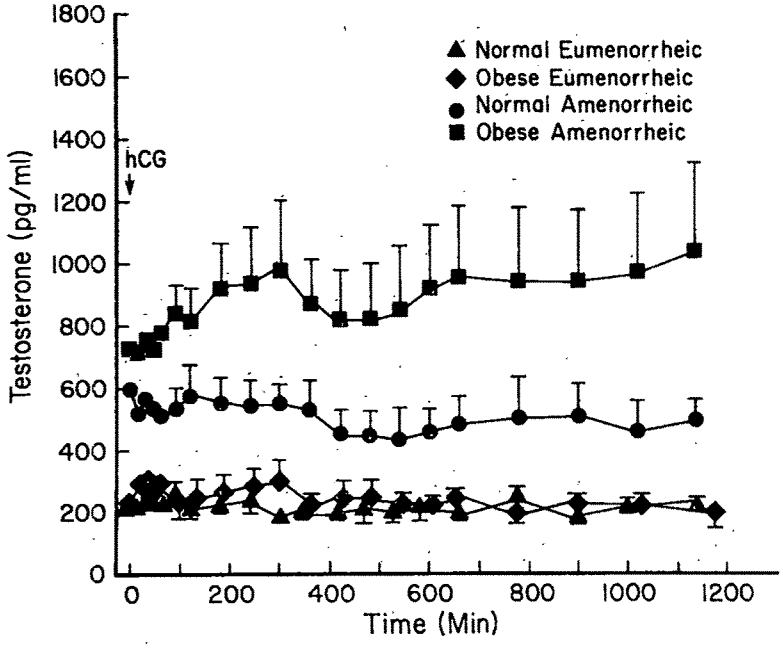


Fig. 4. Changes in serum testosterone over 17 hours in response to 2000 IU/1.5m^2 of intramuscular hCG administered at time zero in eumenorrheic and amenorrheic women further categorized by weight.

the four groups were compared (Table I). Two subjects in the obese anovulatory group, both of whom were extremely obese (173% and 214% ideal body weight, respectively), had particularly high basal immunoreactive insulin values of 67.7 and 77.2 μU/ml. Similarly, the mean maximal rise in immunoreactive insulin levels (maximal changes in insulin) after glucose was elevated ($p < 0.05$) only in obese anovulatory women (Fig. 2). Basal testosterone levels were correlated ($r = 0.48$, $p < 0.05$, Fig. 3) with basal immunoreactive insulin levels.

After hCG administration, testosterone levels rose ($p < 0.005$) in obese anovulatory women only. After 3 hours, testosterone levels had increased by an average

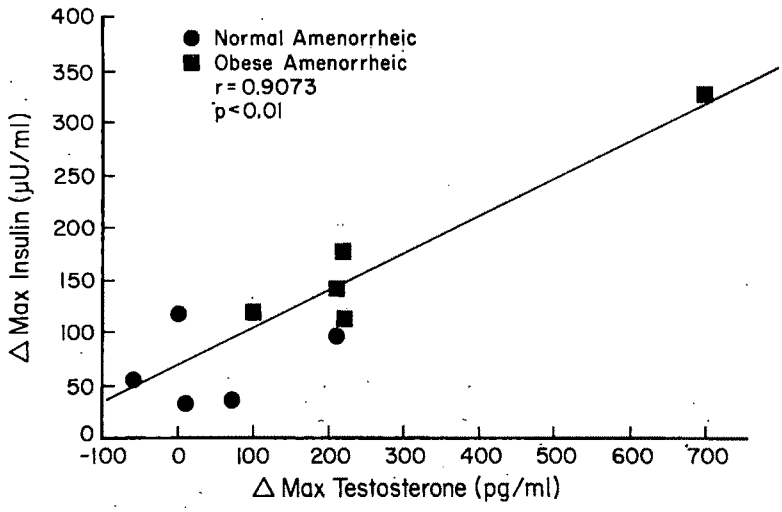


Fig. 5. The relationship between Δ_{max} testosterone over 3 hours after 2000 IU/1.5m² of intramuscular hCG and Δ_{max} insulin over 3 hours after a 100 gm oral glucose load in obese and normal weight amenorrheic women.

of 32% in obese anovulatory women but subsequently remained stable (Fig. 4). The maximal increase in testosterone during the first 3 hours after hCG (change in testosterone) was highly correlated with insulin ($r = 0.91$, $p < 0.01$) in the 10 anovulatory subjects who underwent both studies (Fig. 5).

Comment

It has been recognized for several years that hyperandrogenic, chronically anovulatory women frequently have mild insulin resistance.[2] Because many of these women are also obese, it is possible that the insulin resistance in such persons is simply a reflection of the well-known insulin resistance associated with obesity per se rather than a separate metabolic defect associated with ovulatory dysfunction and independent of body weight. Evidence exists, however, that at least a component of the insulin resistance in this group is not caused by obesity. When a group of obese subjects with polycystic ovary syndrome was compared with a significantly more obese control group, those with polycystic ovary syndrome still had more insulin resistance.[1] Furthermore, insulin resistance has been documented in nonobese subjects with polycystic ovary syndrome.[3] Finally, when both obese and nonobese women with polycystic ovary syndrome were compared with weight-matched control subjects, both polycystic ovary syndrome and obesity were associated separately with the presence of insulin resistance, although obesity seemed to play a much more important role.[12] Our data support the contention that obesity is the primary determinant of insulin resistance in hyperandrogenemic, anovulatory women in general. The fact that the normal weight, anovulatory subjects we studied had no evidence of insulin resistance may be because we did not use the same highly restrictive clinical and biochemical criteria used in almost all the other studies of polycystic ovary syndrome. Specifically, not all of the anovulatory subjects in our study had elevated LH/FSH ratios, perimenarcheal onset of menstrual irregularity, hirsutism, and documented morphologic ovarian abnormalities. Moreover, all but one of the studies cited used immunoreactive insulin responses to oral glucose ingestion as an index of insulin resistance, and it is unlikely that our findings resulted from differences in experimental technique. Our data confirm that chronic anovulation is a heterogeneous condition.

We and others have previously described the presence of hyperandrogenism in anovulatory subjects and its exaggeration in obesity.[12, 13] The present study adds further support to these findings.

Nevertheless, it is possible that basal levels alone are poor indexes of overall androgen production rates. Because we[13] and others[14-16] have shown that both obesity and hyperandrogenism are associated with decreased concentrations of sex hormone–binding globulin and a consequent increase in metabolic clearance rates[17] for circulating androgens, we elected to quantify the degree of LH-dependent ovarian hyperandrogenism in our subjects by administering hCG after dexamethasone suppression and measuring acute changes in testosterone levels. In designing this study, we were aware of data suggesting that hCG does not specifically stimulate the ovary[18, 20]; however, our goal was to suppress adrenal androgen production in all our subjects by the use of overnight dexamethasone suppression and thus minimize any adrenal contribution to the acute change in testosterone levels.

In contrast to basal testosterone data, which were similar in both obese and nonobese anovulatory subjects, the dynamic testing indicated that the obese subjects possessed unique responsiveness to hCG administration. Our data are in agreement with those of others who found correlations between the degree of insulin resistance and testosterone levels.[1-3, 12] The fact that this relationship remained apparent when hCG-stimulated rather than basal testosterone levels were compared with the degree of insulin resistance lends additional support to the hypothesis that the two abnormalities are related. It is important to keep in mind that the fact that a correlation exists between hyperandrogenism and insulin resistance in these subjects does not demonstrate a cause-and-effect relationship. In fact, the persistence of insulin resistance after medical castration suggests that the hyperandrogenism does not induce insulin resistance.[1] Although the elevation in immunoreactive insulin levels associated with insulin resistance may directly induce the ovarian overproduction of androgens, it is still possible that both abnormalities are caused by another as yet undetermined metabolic defect that results in both insulin resistance and an increase in ovarian androgen production. This latter possibility might partly explain why insulin resistance is not always observed in the nonobese anovulatory group.

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Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua

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We examined whether different cell subpopulations from human fetal membranes and decidua produce steroids (estrone and progesterone) and metabolize prostaglandins (prostaglandin $F_{2\alpha}$ to 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ and if these changed with labor. Amnion, chorion, and decidua were obtained at elective cesarean section at term or at spontaneous labor. Cells were dispersed with collagenase and separated by density on discontinuous Percoll gradients. At cesarean section there was a major broad band of cells from amnion and chorion. This band contained most of the estrone sulfatase (estrone sulfate to estrone) activity. The 3β-hydroxysteroid dehydrogenase (pregnenolone to progesterone conversion) and prostaglandin $F_{2\alpha}$ metabolizing activities were present in these cells and those that migrated at greater Percoll densities. Amnion and chorion obtained after spontaneous labor had two major bands of cells. Estrone sulfatase was present in cells from both bands, whereas progesterone output from pregnenolone and prostaglandin $F_{2\alpha}$ metabolism predominated in the second band of cells with greater density. This pattern was particularly apparent in chorion. Dispersed cells from decidua tended to migrate throughout the gradient. In general, estrone sulfate to estrone conversion predominated in lighter cells whereas progesterone output from pregnenolone and prostaglandin $F_{2\alpha}$ metabolism predominated in cells of greater density. The output of progesterone from pregnenolone was significantly lower in cell preparations from chorion and decidua at spontaneous labor compared with cesarean section. We conclude that human amnion, chorion, and decidua contain distinct cell subpopulations based on Percoll migration and that in the membranes these change between cesarean section and spontaneous labor. Partial separation of estrone sulfatase from 3β-hydroxysteroid dehydrogenase and prostaglandin $F_{2\alpha}$ metabolizing activities has been demonstrated, which raises the possibility of paracrine interactions in vivo. (AM J OBSTET GYNECOL 1987;157:1474-81.)

Key words: Amnion, chorion, decidua, steroids, prostaglandins, parturition

Several groups of investigators have shown that human fetal membranes and decidua produce stimulatory prostaglandins (PG) in increasing amounts in association with the onset of human parturition.[1] The amnion and decidua are the major sites of primary PG output, whereas in vitro the chorion produces mainly 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM). In addition, these structures produce steroids from exogenous precursors during late pregnancy and at parturition. We[2] and others[3] have shown that 3β-hydroxysteroid dehydrogenase activity (pregnenolone to progesterone conversion) predominates in the chorion and decidua. The activity of this enzyme decreases in decidua collected after labor of spontaneous onset.[4] Estrone sulfatase activity was also greater in the chorion and decidua compared with the amnion and increased significantly in tissues collected from patients at spontaneous labor compared with tissues collected at elective cesarean section.[5, 6]

Based on these observations, the hypothesis has been proposed that different cell populations in each of amnion, chorion, and decidua produce steroids and PGs, thereby allowing for paracrine interactions within these tissues in the maintenance of pregnancy and in the stimulus to birth. To explore this hypothesis, we have separated cell populations from amnion, chorion, and decidua on Percoll density gradients and examined whether the same or different cell populations in these tissues convert pregnenolone to progesterone (3β-hydroxysteroid dehydrogenase $\delta^{4,5}$ isomerase activity), estrone sulfate to estrone (estrone sulfatase activity), and $PGF_{2\alpha}$ to PGFM (15-hydroxyprostaglandin dehy-

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drogenase $-\delta^{13,14}$ reductase activity). In addition, we have examined whether the cell populations showing these activities might change at the time of labor. Our results suggest that in amnion, chorion, and decidua, the steroid and PG metabolic activities are located in part in different cell subpopulations based on Percoll density separation. The distribution of cells changes between cesarean section and spontaneous labor, particularly in amnion and chorion.

Material and methods

Patients. The fetal membranes and adherent decidual tissue were collected from six patients at elective cesarean section at term without labor and from seven patients with term labor of spontaneous onset. In four of these women, tissues were collected after spontaneous vaginal delivery, and in three patients, the tissues were collected at cesarean section in labor. For analysis, results from these seven patients have been pooled. None of the patients received oxytocics.

Tissue preparation. The amnion was gently peeled away from the chorion. Decidua was scraped off the chorion as described previously.[7] Chorion and decidual tissue can be obtained in this way with <10% to 15% contamination. The tissues were then minced and dispersed into isolated cell preparations with the use of 0.05% collagenase and 0.003% deoxyribonuclease essentially as previously described.[7]

The mixed cells (2 ml suspension) were layered onto a discontinuous Percoll gradient, which was prepared essentially as described by Gibb and Lavoie,[8] in Krebs-Ringer bicarbonate buffer containing 1% albumin. The gradients were prepared in glass tubes to which successive 2.5 ml volumes of 60%, 40%, 20%, and 5% Percoll were added. The samples were centrifuged at 800 × g for 20 minutes at 4° C. After centrifugation, consecutive 1 ml fractions were collected from the top of the gradient to the bottom of the tube. A single drop was taken from each fraction to determine the density with a refractometer. The cells were centrifuged and washed three times at 4° C in Krebs-Ringer buffer containing 1% albumin. After the third wash, the cells were resuspended in 2 ml Krebs-Ringer bicarbonate buffer. An aliquot of cells was diluted with Hematall isotonic diluent, treated with 0.3% potassium cyanide (Hematall LA-Hgb reagent, Fisher Diagnostics, Orangeburg, New York) to lyse red blood cells, and then counted with a Coulter counter. Preliminary studies established that similar cell counts were obtained with a Coulter counter and by direct counting with a hemocytometer. Viability was assessed by the ability of the cells to exclude Trypan blue dye and was generally >85% to 90%. In preliminary experiments we established that single cell suspensions, which were essentially free of any aggregates, were present in mixed cell preparations and individual subpopulations obtained after Percoll gradient centrifugation.

An aliquot (500 μl) of the washed cells from each fraction off the Percoll column was then added to glass tubes containing pregnenolone (Steraloids Ltd.) or estrone sulfate (kindly provided by Dr. R. Hobkirk, Department of Biochemistry, University of Western Ontario), or $PGF_{2\alpha}$ (Upjohn Co., Ltd.). All three substrates were added at a concentration of 100 ng/100 μl of Krebs-Ringer bicarbonate buffer containing 1% albumin. The tubes were then incubated for 4 hours at 37° C in a shaking water bath. At the end of this period, the incubates were frozen and stored at −20° C until subsequent analysis. Progesterone, estrone, and PGFM were measured later in 0.5 ml aliquots by radioimmunoassays that have been described and validated previously.[2, 5, 7] Samples were acidified with citric acid before extraction of PGFM. PGFM and estrone were extracted twice with five volumes of diethyl ether; progesterone was extracted twice with five volumes of petroleum ether. Extraction recoveries were calculated by means of parallel tubes with the addition of approximately 1000 cpm [^{3}H] PGFM, progesterone, or estrone, as appropriate. Extraction efficiency was generally >85%, and all measurements were corrected for within assay recovery. The specificity and coefficients of variation for these assays on media from incubates of dispersed cells or explants of human fetal membranes and decidua have been previously described.[2, 5, 9] The combined inter- and intra-assay coefficients of variation in the present study ranged between 10% and 17%. Blank tubes containing the substrates pregnenolone, estrone sulfate, or $PGF_{2\alpha}$ but no cells were included in each incubation. The radioimmunoassay values in these tubes were subtracted from those in tubes containing cells to correct for cross-reactivities of the substrates in the different radioimmunoassays. The conversions to progesterone, estrone, and PGFM were expressed as net output per milliliter elution fraction from the Percoll column or as picograms per 10^5 cells for a 4-hour incubation period. Cell counts were made, and similar incubations were conducted in the presence of the different substrates on an aliquot of the mixed cell populations (100% cell fraction) before application to the Percoll columns.

Analysis of results. The output of progesterone, estrone, and PGFM was calculated for successive 1 ml elution fractions from the Percoll column. The sum of the separate cell counts in the different column fractions was expressed as a percentage of the total cells applied to the column to assess the losses of cells during the separation and washing procedures. The mean recovery of cells for all patients was 45.4% ± 5.7%, 45.4% ± 7.7%, and 34.3% ± 4.9% (SEM; n = 13) from amnion, chorion, and decidua, respectively. Be-

Table I. Net accumulation of estrone, progesterone, and PGFM by initial 100% cell fractions obtained before gradient separation

Tissue	*Product*	*Cesarean section (n = 6)*	*Spontaneous labor (n = 7)*
Amnion	Estrone	1.44 ± 0.24* (27.54)	1.65 ± 0.21 (44.67)
	Progesterone	1.87 ± 0.32 (74.13)	1.93 ± 0.26 (85.11)
	PGFM	1.12 ± 0.12 (13.18)	1.41 ± 0.21 (25.70)
Chorion	Estrone	2.39 ± 0.14 (245.47)	2.74 ± 0.20 (549.54)
	Progesterone	3.64 ± 0.25 (4365.16)	2.63 ± 0.34† (426.58)
	PGFM	2.43 ± 0.33 (269.15)	1.73 ± 0.31 (53.70)
Decidua	Estrone	1.86 ± 0.19 (72.44)	2.07 ± 0.17 (117.49)
	Progesterone	3.29 ± 0.22 (1949.84)	2.65 ± 0.17† (446.68)
	PGFM	1.99 ± 0.19 (97.72)	1.46 ± 0.35 (28.84)

*Results are given as log picograms per 10^5 cells per 4 hours (mean ± SEM). Geometric means are given in parentheses.
†$p < 0.05$ versus cesarean section group.

Table II. Sum of net accumulation of estrone, progesterone, and PGFM by separated cell fractions of 1 ml eluates from Percoll columns

Tissue	*Product*	*Cesarean section (n = 6)*	*Spontaneous labor (n = 7)*
Amnion	Estrone	1.64 ± 0.27* (43.65)	1.98 ± 0.24 (95.49)
	Progesterone	2.16 ± 0.30 (144.54)	2.38 ± 0.38 (239.88)
	PGFM	2.09 ± 0.23 (123.02)	2.12 ± 0.33 (131.83)
Chorion	Estrone	2.44 ± 0.28 (275.42)	2.54 ± 0.31 (346.74)
	Progesterone	4.11 ± 0.15 (12882.50)	2.90 ± 0.51† (794.33)
	PGFM	2.58 ± 0.31 (380.19)	2.56 ± 0.32 (363.08)
Decidua	Estrone	1.89 ± 0.35 (77.62)	2.21 ± 0.30 (162.18)
	Progesterone	3.77 ± 0.28 (5888.44)	2.65 ± 0.41† (446.68)
	PGFM	2.47 ± 0.28 (295.12)	2.00 ± 0.36 (100.00)

*Results are given as log picograms per 10^5 cells per 4 hours (mean ± SEM). Geometric means are given in parentheses.
†$p < 0.05$ versus cesarean section group.

cause of the variability in cell counts between patients, the cell elution profiles were normalized by expressing the cell count in each fraction as a percentage of the cell count in the Percoll fraction with the maximum number of cells.

In some experiments the sum of progesterone, estrone, or PGFM output from the separate column fractions was calculated, corrected for the recovery of cells, and compared with the output of progesterone, estrone, and PGFM by the mixed cell populations. The output of progesterone, estrone, and PGFM in the initial 100% cell fractions and the sum of hormone outputs in the separated Percoll fractions were expressed per 10^5 cells. Comparison between the cesarean section and spontaneous labor groups was made by the Student *t* test after logarithmic transformation to correct for heterogeneity of variance.

Results

Net output of estrone, progesterone, and PGFM. The net accumulation of estrone, progesterone, and PGFM from estrone sulfate, pregnenolone, and $PGF_{2\alpha}$, respectively, during 4-hour incubations by the mixed cell preparations of different tissues is shown in Table I. Estrone output by amnion cells was less than that for chorion or decidua and did not change significantly with labor. The mean output of estrone from estrone sulfate by chorion and decidual cells increased with cells from patients in labor, although these differences were not statistically significant. The net accumulation of progesterone from pregnenolone was greater in chorion and decidua than in amnion. There was a significantly lower conversion of pregnenolone to progesterone by cell preparations of chorion and decidua obtained from patients at active labor compared with the conversion of pregnenolone to progesterone by these tissues at elective cesarean section ($p < 0.05$). The mean output of PGFM from $PGF_{2\alpha}$ by chorion cells was greater than that from amnion and decidua. There was no change in the output of PGFM From $PGF_{2\alpha}$ in cells from any tissue as a function of labor.

The effect of labor on estrone, progesterone, and PGFM output was also sought from the sum of separate conversions by the different fractions from the Percoll columns (Table II). In general, the results were similar to those obtained with the mixed cell preparations, although product formation per 10^5 cells tended to be higher. There was a significant decrease in the output of progesterone from pregnenolone by chorion and decidual cell preparations obtained after labor compared with the conversions by cells obtained from patients at elective cesarean section at term but not in labor (Table II; $p < 0.05$).

Elution profiles on Percoll

Amnion. The cells from amnion obtained at elective cesarean section eluted with a broad band of densities, ranging from 1.014 to 1.020 (Fig. 1, *a*). Cells in these fractions contained most of the estrone sulfatase activity. In contrast, cells forming progesterone from pregnenolone and PGFM from $PGF_{2\alpha}$ were distributed

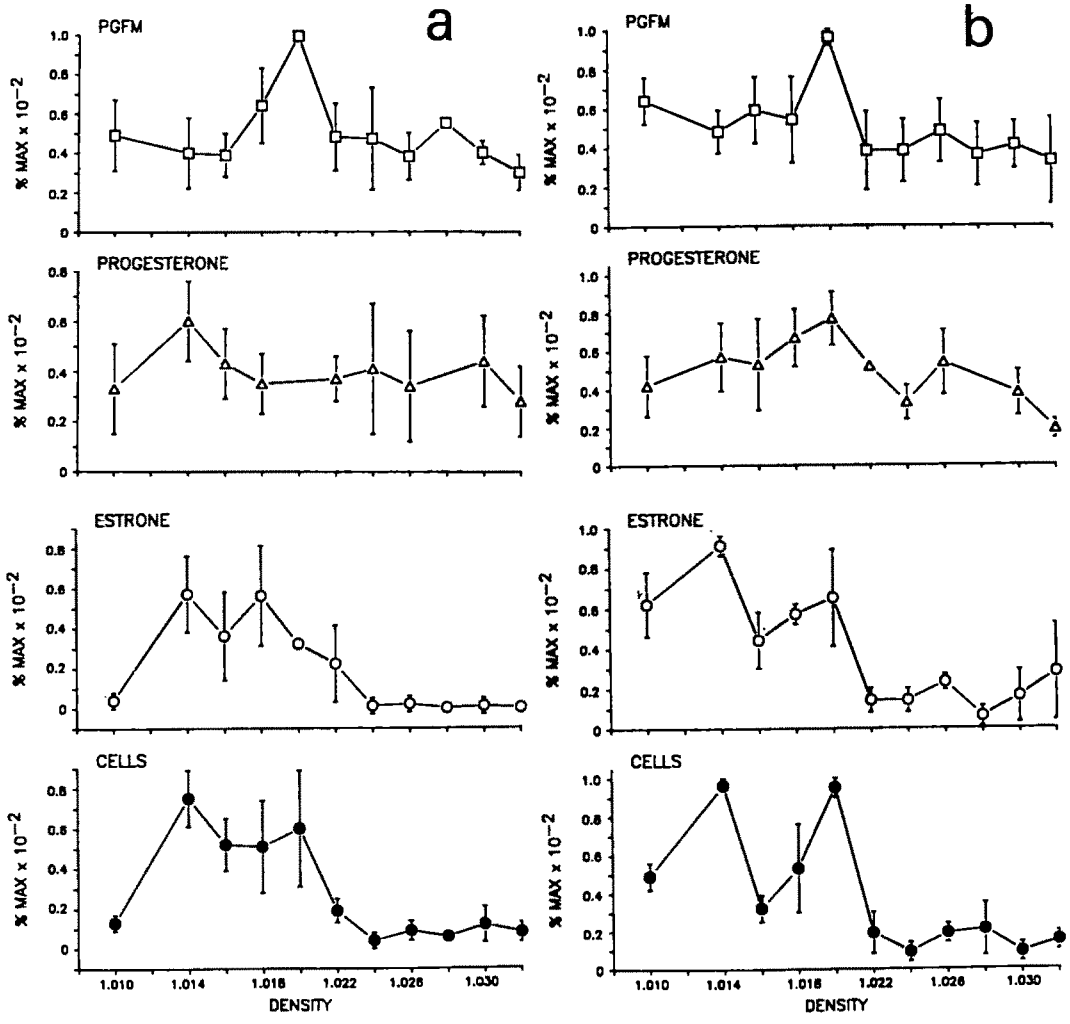


Fig. 1. The elution profiles on Percoll density gradients of cells (●) and PGFM formed from $PGF_{2\alpha}$ (□), progesterone formed from pregnenolone (△), and estrone formed from estrone sulfate (○) by amnion obtained from patients at term elective cesarean section **(a)** and at term spontaneous labor **(a)**. Values are mean ± SEM for six and seven patients, respectively.

throughout the density gradient. Cells from amnion obtained from patients in labor eluted with two major density peaks at 1.014 and 1.020 (Fig. 1, *b*). In general, the output of estrone and estrone sulfate, followed the same patten as cell distribution, with relatively little activity in cells migrating at densities >1.022. The cell bands that showed the highest conversion of pregnenolone to progesterone and $PGF_{2\alpha}$ to PGFM eluted at a density of 1.020. However, both of these activities were present in other cell fractions, which were distributed throughout the column (Fig. 1, *b*).

Chorion. Dispersed cells from chorion obtained from patients not in labor migrated predominantly as a single peak at densities of 1.012 to 1.014 (Fig. 2, *a*). Conversion of estrone sulfate to estrone was greatest by cells in this fraction. In contrast, the net accumulation of progesterone and PGFM from pregnenolone and $PGF_{2\alpha}$, respectively, was higher in cell fractions with greater densities.

Dispersed cells from chorion obtained at labor migrated with two major subpopulations at densities of 1.014 and 1.020, although smaller numbers of cells were eluted at other densities. The conversion of estrone sulfate to estrone followed a pattern similar to the elution profile of the cells, and the output of estrone was greatest in cell peaks at densities of 1.014 and 1.020. The output of progesterone from pregnenolone and PGFM from $PGF_{2\alpha}$ was greatest in cells that migrated at a density of 1.020, but these activities were also present in cells that migrated at heavier densities (Fig. 2, *b*).

Decidua. Decidual cells obtained at cesarean section migrated throughout the Percoll gradients. The output of estrone and estrone sulfate tended to be greater in

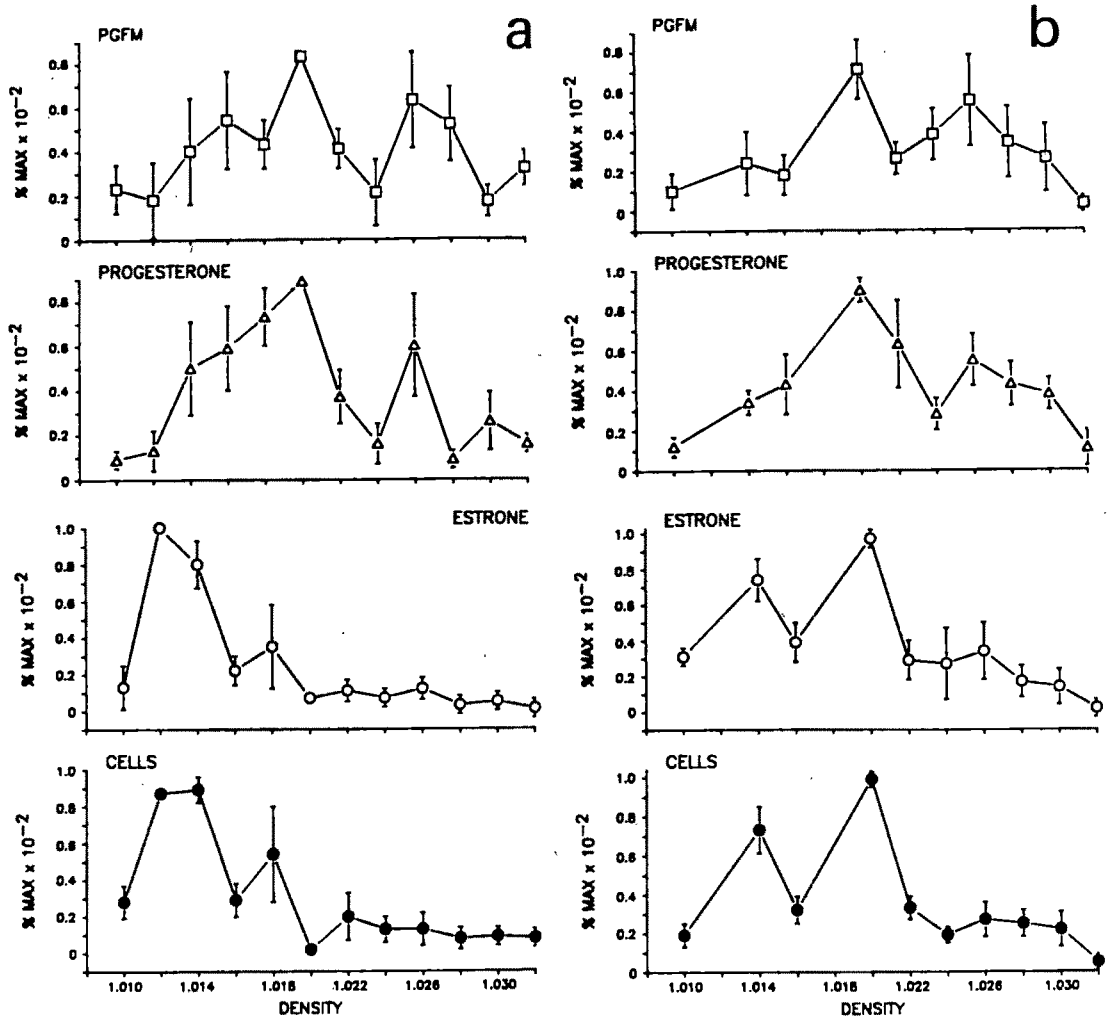


Fig. 2. The elution profiles on Percoll density gradients of cells (●) and PGFM formed from $PGF_{2\alpha}$ (□), progesterone formed from pregnenolone (△), and estrone formed from estrone sulfate (○) by chorion obtained from patients at term elective cesarean section **(a)** and at term spontaneous labor **(b)**. Values are mean ± SEM for six and seven patients, respectively.

the less dense fractions (1.012 to 1.018), whereas the net accumulation of progesterone or PGFM was greater in cells that migrated at higher densities (1.018 to 1.026). In general, the output of progesterone from pregnenolone and $PGF_{2\alpha}$ from PGFM showed a similar elution profile (Fig. 3, *a*). Decidual cells obtained from tissues at spontaneous labor were also distributed throughout the Percoll columns. The net accumulation of estrone from estrone sulfate was most evident in fractions of 1.010 to 1.020. The net output of estrone from estrone sulfate was less in the denser cell fractions. In contrast, there was little formation of progesterone or PGFM in the three lightest cell fractions. The net outputs of progesterone and PGFM were greatest in coincident subpopulations at a density of 1.020, although these products were also formed in all other cell fractions migrating at greater densities.

Comparison of hormone output in the mixed and separated cell fractions. The formation of estrone, progesterone, and PGFM by the separated cell fractions after correcting for cell losses through the column was compared with the output of these products in the initial 100% cell preparations. Although there was considerable variability between patients, the output of estrone from estrone sulfate was as much as 2.5 times greater in the separated cell preparations, whereas the average output of progesterone from pregnenolone and PGFM from $PGF_{2\alpha}$ was three to five times greater as the sum of the separated cell fractions (Table III). The differences between PGFM output by chorion ob-

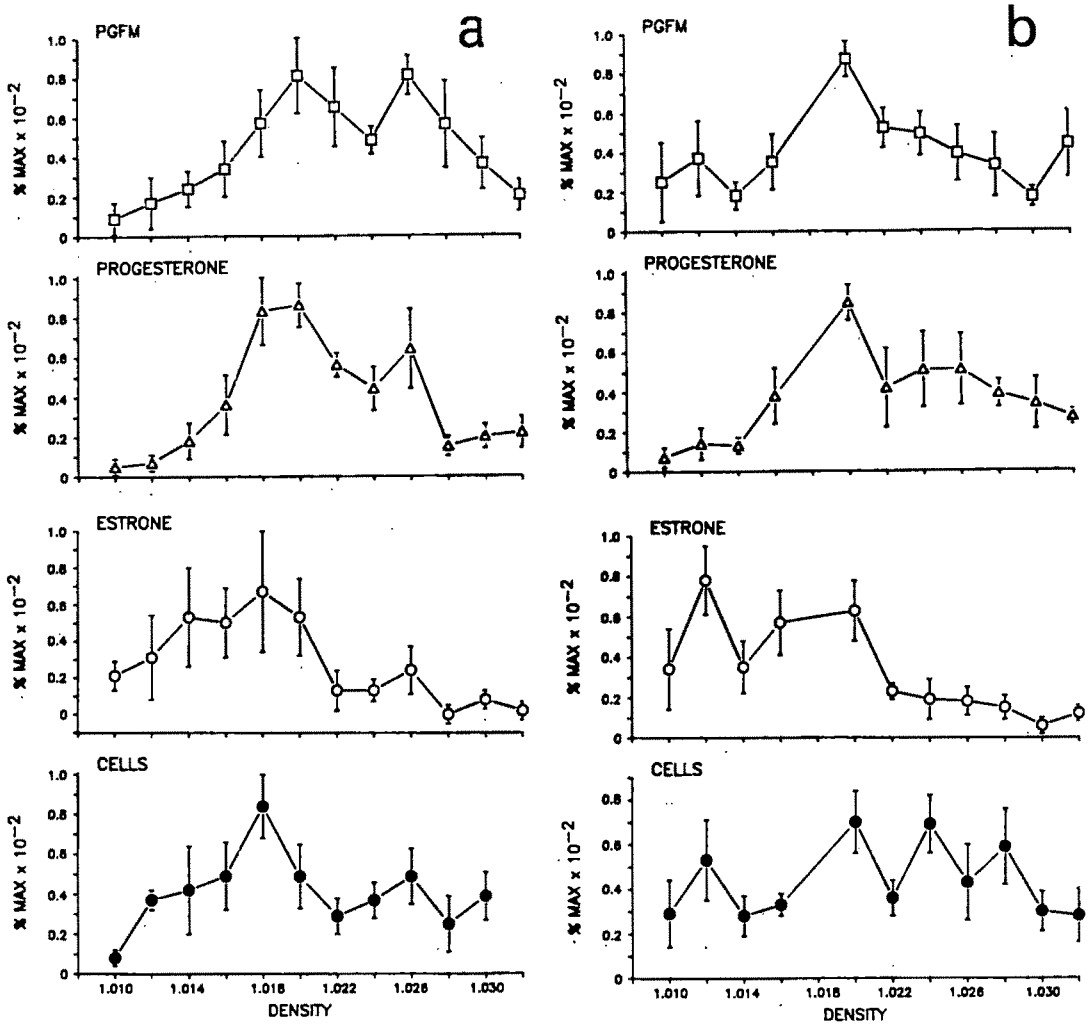


Fig. 3. The elution profiles on Percoll density gradients of cells (●) and PGFM formed from $PGF_{2\alpha}$ (□), progesterone formed from pregnenolone (△), and estrone formed from estrone sulfate (○) by decidua obtained from patients at term elective cesarean section (a) and at term spontaneous labor (a). Values are mean ± SEM for six and seven patients, respectively.

tained at elective cesarean section and spontaneous labor were significant ($p < 0.02$; t test).

Comment

The present study provides evidence that different cell populations based on density gradient separation can be obtained from human amnion, chorion, and decidua. In the membranes, the distribution of these cell subpopulations changes between cesarean section and spontaneous labor. Moreover, we found partial separation between cell fractions in the ability to convert estrone sulfate to estrone, pregnenolone to progesterone, and $PGF_{2\alpha}$ to PGFM. In addition, the net output of progesterone from pregnenolone by chorion and decidual cells decreased in preparations from women in active labor compared with patients at cesarean section at term but not in labor.

We have used Percoll to separate cell subpopulations. Preliminary studies established that individual cells without aggregates were present in the different fractions from the gradients. Differences between the fractions are attributable to differences in cell density, and the changes with labor are likely caused by an increase in cellular granular material or other intracellular inclusions rather than the appearance of a novel cell type. Further studies will require morphologic assessment of these subpopulations and the use of other physical methods of separation. After Percoll separation, cell viability was good, as indicated by the exclusion of Trypan blue dye. The differences in cell populations are

Table III. Ratio of the net accumulation of estrone, progesterone, and PGFM from exogenous substrates by the sum of separated cell fractions after Percoll with that by initial 100% cell fractions before Percoll

Tissue	*Product*	*Cesarean section (n = 6)*	*Spontaneous labor (n = 7)*
Amnion	Estrone	2.93 ± 1.31*	1.25 ± 0.23
	Progesterone	3.27 ± 0.87	4.72 ± 0.84
	PGFM	5.44 ± 1.71	8.29 ± 2.17
Chorion	Estrone	2.49 ± 1.28	1.89 ± 0.66
	Progesterone	2.74 ± 1.12	4.11 ± 1.19
	PGFM	2.13 ± 0.67	5.61 ± 1.05†
Decidua	Estrone	2.80 ± 1.36	2.21 ± 0.57
	Progesterone	3.45 ± 0.64	5.15 ± 1.75
	PGFM	4.82 ± 0.77	3.65 ± 0.83

*All values are mean ± SEM for the number of patients indicated; conversions to products are E_1S to estrone, pregnenolone to progesterone, and $PGF_{2\alpha}$ to PGFM.

†$p < 0.02$ versus cesarean section.

not likely to be the result of mechanical trauma because viability and cell recovery were similar with or without labor. Membranes collected from three patients at cesarean section in labor showed a distribution of cell subpopulations similar to that from four patients from whom tissue was collected after labor of spontaneous onset. Although the Percoll columns were set up as discontinuous gradients,[8] they were not maintained after centrifugation, and the results have been expressed to reflect the Percoll densities in successive 1 ml fractions containing cells.

The present study demonstrated the potential for precursor uptake and interconversion by intact cell preparations. We recognize that limitations of substrate and cofactor availability preclude calculation of enzyme kinetic parameters. However, in other studies we have shown the time and substrate concentration dependence of steroid conversions by intact cells. Moreover, net conversions even at substrate concentrations less than the Michaelis constant (K_m) of estrone sulfatase by intact cells have reliably predicted differences in enzyme activities between tissues obtained from patients at cesarean section or spontaneous labor.[5, 6, 10, 11]

The conversion of pregnenolone to progesterone was lower in initial 100% cell preparations and the sum of the separate cell fractions from chorion and decidua obtained at spontaneous labor compared with that at elective cesarean section at term. A similar decrease in pregnenolone to progesterone conversion at spontaneous labor has been reported with decidual explants.[1] These observations suggest that 3β-hydroxysteroid dehydrogenase $\delta^{4,5}$ isomerase (3β-hydroxysteroid dehydrogenase) activity may be reduced in these tissues at labor, which raises the possibility of local withdrawal of progesterone production from these intrauterine sources. Previous reports have shown that the activity of 3β-hydroxysteroid dehydrogenase is greater in chorion than in decidua or amnion,[2] which was confirmed in the present study. The mean conversion of pregnenolone to progesterone was greater in the sum of separated cell subpopulations than in the mixed cell preparations, which would be consistent with removal of an endogenous inhibitor by Percoll separation. Estrogens could be such inhibitors.[2, 12] The conversion of pregnenolone to progesterone in mixed cells is inhibited by estrone and estradiol,[2] and the major cell subpopulations converting estrone sulfate to estrone were separated from those converting pregnenolone to progesterone in the present study.

We have previously reported that the mean conversion of estrone sulfate to estrone[5] and estrone sulfatase activity[6] rises in chorion and decidua with labor. A similar trend was noted in the present study, although statistical significance was not achieved. Previous reports have indicated that estrone sulfatase activity is greater in chorion and decidua,[5] although activity is present in amnion.[5, 13] A similar distribution of estrone sulfatase was found in the present study. Steroid sulfatase activity with dehydroepiandrosterone sulfate as a substrate was not found in amnion, although is present in chorion.[8, 13, 14]

We also confirmed the very low conversion of $PGF_{2\alpha}$ to PGFM (15-hydroxyprostaglandin dehydrogenase) in amnion and greater output of PGFM in chorion.[15] It was previously reported that the output of PGFM by chorion cells from endogenous precursors increased at the time of labor.[7] The present study suggests that this is likely caused by increased formation of $PGF_{2\alpha}$ in chorion, since our results indicate that the conversion of $PGF_{2\alpha}$ to PGFM did not appear to change with the onset of labor. In general, the output of PGFM from $PGF_{2\alpha}$ was greater as the sum of activities from separated cell subpopulations compared with the mixed cell fractions, which might suggest that this conversion is influenced by an inhibitory component. The potential exists for 15-hydroxyprostaglandin dehydrogenase regulation during pregnancy. 15-Hydroxyprostaglandin dehydrogenase in the fetal lung[16, 17] and uterus[18] of a rabbit increases during pregnancy. It is possible that the $PGF_{2\alpha}$ to PGFM conversion is influenced by steroids of pregnancy, including progesterone, and is also regulated in the mixed cell preparations of human fetal membranes in vitro.

This study is the first report to separate cell subpopulations from human fetal membranes and decidua that convert estrone sulfate to estrone from cell populations that convert pregnenolone to progesterone and $PGF_{2\alpha}$ to PGFM. Estrone sulfatase activity was present in cells migrating at lighter densities, whereas pro-

gesterone and PGFM formation from pregnenolone and $PGF_{2\alpha}$, respectively, were present more in cells that migrated at greater density. In general, cells converting pregnenolone to progesterone and $PGF_{2\alpha}$ to PGFM migrated with similar densities, although the present study does not reveal whether the same or different cell groups are involved in those conversions. We expressed our results on the basis of successive fractions from the Percoll gradient and did not establish whether the enzymatic conversions were attributable to all the cells in any one subpopulation or to a variable percentage of them. Gibb and Lavoie[8] expressed their results in a similar manner and also showed that 3β-hydroxysteroid dehydrogenase activity in chorion cells was distributed widely through the gradient. In that report, dehydroepiandrosterone sulfatase activity was measured and was found to predominate in lighter cells, resembling the pattern with estrone sulfatase activity in the present study.

The significance of the change in distribution of cell populations with labor requires further study. It is of interest that the second band of cells in the chorion and amnion actively convert estrone sulfate to estrone, and this activity increases with labor.[5, 6] The results of the present report raise clearly the possibility of paracrine or autocrine interactions between cell types in amnion, chorion, and decidua. Steroids affect prostaglandin cyclooxygenase and 15-hydroxyprostaglandin dehydrogenase activities,[18] and estrogen may inhibit 3β-dehydroxysteroid dehydrogenase activity.[12] Furthermore, the potential for local increases in estrogen output and decreases in progesterone formation from pregnenolone raises the possibility that these may occur in the absence of alterations in circulating steroid hormone concentrations.[19] Such changes may exert influences on agonist receptor populations and gap junction formation and thereby contribute to pregnancy maintenance and birth.

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Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: Fetal plasma concentrations and cardiorespiratory changes

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Tocolytic doses of nicardipine, a dihydropyridine calcium entry blocker, were administered to chronically catheterized rhesus monkeys between days 128 and 132 of gestation. During periods of spontaneous uterine contractility, a 500 μg nicardipine bolus was injected intravenously, and this was followed by continuous infusion (6 μg/kg/min) to the mother for 1 hour. Uterine activity (amniotic fluid pressure) and maternal heart rate and blood pressure were monitored continuously. Paired maternal and fetal blood samples were drawn at frequent intervals to monitor pH, Po_2, Pco_2, and plasma nicardipine concentrations. Peak maternal nicardipine concentrations ranged from 175 to 865 ng/ml while peak fetal levels ranged from 7 to 35 ng/ml. Fetal heart rate and blood pressure were unaffected. However, fetuses became acidotic (pH 7.26 ± 0.01 versus 7.33 ± 0.01) and hypoxemic (Po_2 16.0 ± 3.2 versus 24.5 ± 2.0 mm Hg) after maternal nicardipine treatment ($p < 0.01$). Despite the fact that maternal nicardipine treatment exerted a significant tocolytic effect, the undesirable fetal side effects are of concern and deserve further investigation. (Am J Obstet Gynecol 1987;157:1482-6.)

Key words: Uterine contractility, nicardipine, fetus, rhesus monkey

Calcium entry blockers are potent smooth muscle relaxants and are widely used clinically for treatment of cardiovascular disorders.[1] More recently, a number of reports have suggested the potential use of calcium entry blockers as uterine tocolytic agents.[2, 3]

Little information is currently available regarding the effect of tocolytic doses of calcium entry blockers on fetal or neonatal well-being. Limited studies in the human have failed to detect any major adverse effects of maternal administration of the dihydropyridine calcium entry blockers on neonatal outcome.[4, 5] However, delivery of viable fetuses with normal Apgar scores is only a gross indicator of fetal safety. The extent of transplacental passage of calcium entry blockers and their fetal metabolic or hemodynamic effects have not been adequately evaluated in human pregnancy for obvious ethical reasons.

More complete animal studies have documented a beneficial tocolytic effect of nifedipine and nicardipine. However, a number of these studies have also revealed a wide range of deleterious side effects. Decreases in maternal arterial pressure, uterine blood flow, fetal arterial pressure, and fetal-oxygenation have been reported in studies in pregnant rabbits and sheep.[6-8] Experiments in the pregnant rhesus monkey have demonstrated a significant compromise of fetal oxygenation when tocolytic doses of nifedipine were administered to the mother.[9] The effects of nicardipine, a calcium entry blocker that also has a potent phosphodiesterase inhibitory action, have not been studied previously in a primate animal model.

The present study was designed to extend our observations on the fetal cardiorespiratory effects of maternal calcium entry blocker administration and to quantitate the transplacental passage of nicardipine in the rhesus macaque during late gestation.

Material and methods

Operation. Four rhesus monkeys *(Macaca mulatta)* between 128 and 132 days' gestation (term = 167 days) were used in the present study. Surgical procedures were performed by methods previously described in detail.[10] Briefly, with the animals under halothane anesthesia, polyvinyl catheters were implanted in the ma-

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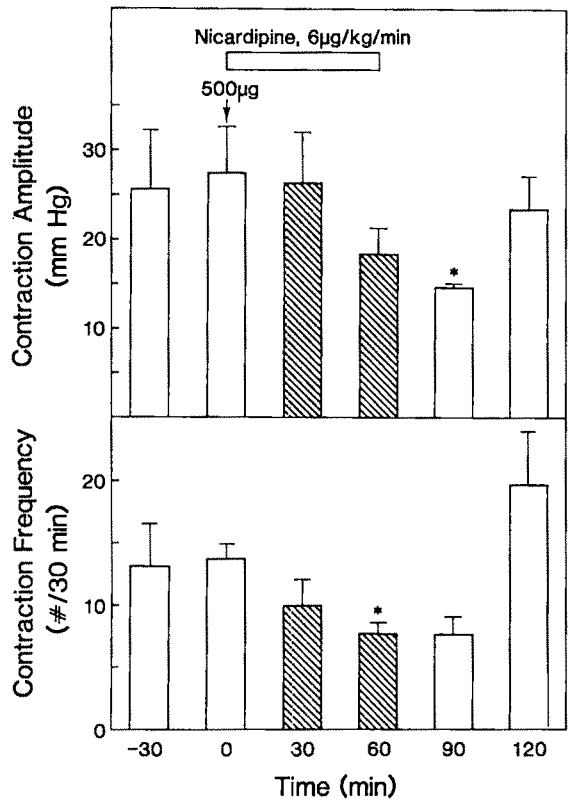


Fig. 1. Effect of maternal nicardipine administration on mean contraction amplitude and contraction frequency. *Cross-hatched bars* (t_{30}, t_{60}) represent sampling intervals during maternal nicardipine treatment. All values are mean ± SEM (n = 4). *Asterisk* indicates significant difference from control values (t_{-30}, t_0) ($p < 0.05$).

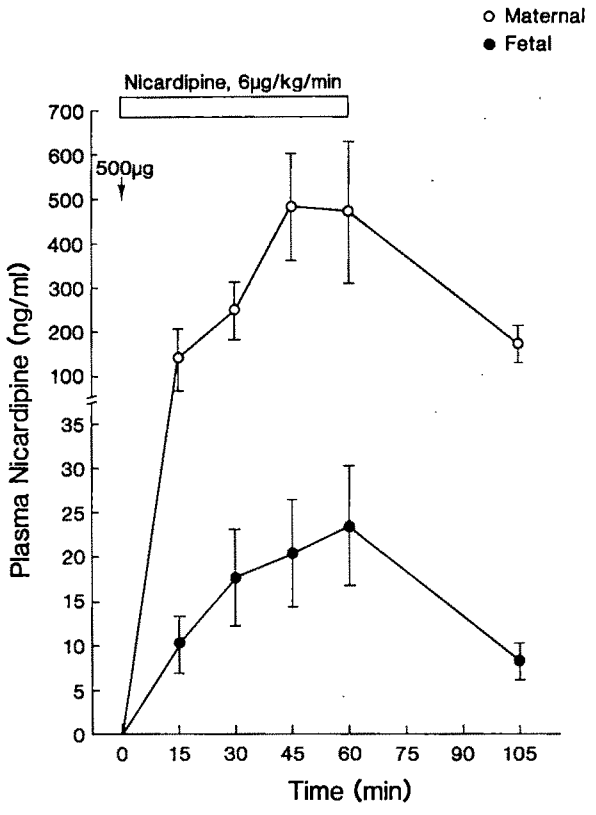


Fig. 2. Maternal and fetal plasma nicardipine concentrations after maternal nicardipine administration. All values represent mean ± SEM (n = 4). Maternal nicardipine (500 μg bolus followed by 6 μg/kg/min intravenous infusion) treatment was begun at t_0 and discontinued at t_{60}.

ternal femoral artery and vein, the fetal carotid artery and jugular vein, and the amniotic fluid cavity. Electrocardiogram electrodes were also attached to the fetus.

Study protocol. Three to four days after operation, during periods of spontaneous uterine contractility, a 500 μg bolus of nicardipine hydrochloride (Syntex Laboratories, Palo Alto, California) in buffer was injected between uterine contractions into the maternal venous catheter, and this was followed by a continuous infusion (6 μg/kg/min) for 1 hour by a Harvard infusion pump. Uterine contractility (amniotic fluid pressure) and fetal and maternal heart rate and arterial pressure were monitored continuously throughout the study. Paired fetal and maternal arterial and amniotic fluid samples were collected at 15- to 30-minute intervals 1 hour before, during, and 1 hour after maternal nicardipine administration. Maternal and fetal arterial pH, P_{CO_2}, and P_{O_2} were determined with a Radiometer blood gas analyzer. Samples for nicardipine quantitation were stored at −70° C until analysis by a modified capillary gas chromatography–electron-capture detection method.[11]

Statistical analysis. Differences between means of control and experimental measurements were tested by two-way analysis of variance and Duncan's multiple-range test. Values are expressed as means ± SEM.

Results

Uterine contractility. The effect of maternal nicardipine administration on uterine contractility is illustrated in Fig. 1. Mean contraction amplitude decreased within 30 to 60 minutes after nicardipine infusion was started and was significantly lower ($p < 0.05$) than control values by 90 minutes after the beginning of treatment (Fig. 1). Contraction frequency followed a similar pattern in which values declined from a control mean of 15.0 ± 2.5 to 10.1 ± 1.9 contractions per 30 minutes at 30 minutes after the start of the nicardipine infusion (Fig. 1). Values declined further at 60 minutes to 7.5 ± 1.5 contractions per 30 minutes, which was a statistically significant change from the control value ($p < 0.05$). The cumulative tocolytic effect was apparent at the end of the nicardipine infusion and was sustained for 30 minutes after the infusion was terminated. The effect was transient, however, since values for both

Table I. Maternal and fetal mean arterial pressure and heart rate in response to maternal nicradipine administration

Time	*Mean arterial pressure (mm Hg)*		*Heart rate (bpm)*	
	Maternal	*Fetal*	*Maternal*	*Fetal*
−30	93.6 ± 1.7	33.1 ± 0.6	181.3 ± 13.5	205.7 ± 11.4
0	93.6 ± 1.7	32.3 ± 0.2	176.3 ± 10.8	200.9 ± 5.7
30	90.0 ± 3.6	32.1 ± 0.5	183.3 ± 9.3	198.0 ± 9.1
60	84.0 ± 2.1*	31.0 ± 0.3	193.0 ± 13.3	204.3 ± 8.6
90	84.3 ± 4.3	29.7 ± 1.1	183.3 ± 12.0	207.4 ± 10.0
120	84.7 ± 3.7	30.9 ± 1.1	184.0 ± 7.0	201.2 ± 7.1

All values represent mean ± SEM (n = 4).
*Significantly different from control (t_{-30}, t_0): $p < 0.05$.

Table II. Maternal and fetal arterial pH and P_{CO_2} in response to maternal nicardipine administration

Time	*pH*		*P_{CO_2} (mm Hg)*		*P_{O_2} (mm Hg)*	
	Maternal	*Fetal*	*Maternal*	*Fetal*	*Maternal*	*Fetal*
−30	7.455 ± 0.039	7.331 ± 0.009	24.7 ± 2.1	35.5 ± 2.4	103.7 ± 8.9	24.9 ± 2.4
0	7.466 ± 0.029	7.334 ± 0.011	23.6 ± 2.5	36.7 ± 1.0	107.6 ± 10.8	24.2 ± 1.7
30	7.458 ± 0.026	7.301 ± 0.006*	26.4 ± 0.8	42.7 ± 3.7	101.8 ± 8.0	18.3 ± 3.6†
60	7.466 ± 0.025	7.280 ± 0.007†	26.4 ± 1.1	44.7 ± 3.5*	101.1 ± 10.4	16.0 ± 3.2†
90	7.453 ± 0.029	7.268 ± 0.016†	26.1 ± 1.3	45.2 ± 4.2*	106.2 ± 10.6	16.8 ± 2.2†
120	7.450 ± 0.028	7.280 ± 0.016†	25.3 ± 2.4	40.8 ± 5.4	102.1 ± 11.7	19.6 ± 4.0†

All values represent mean ± SEM (n = 4).
*Significantly different from control (t_{-30}, t_0): $p < 0.05$.
†$p < 0.01$.

mean contraction amplitude and contraction frequency returned to control levels within 60 minutes after discontinuing nicardipine (Fig. 1).

Maternal and fetal hemodynamics. Mean maternal arterial pressure declined significantly ($p < 0.05$) from control levels of 93.6 ± 1.7 to 84.0 ± 2.1 mm Hg at 60 minutes (Table I). Pressure remained depressed at 90 and 120 minutes but the difference was not significant. Compensatory maternal tachycardia was observed with an elevation in maternal heart rate at 60 minutes that was not significant (Table I). Values returned to control levels in the hour after nicardipine infusion. A slight but nonsignificant decline in fetal mean arterial pressure was observed after maternal nicardipine infusion (Table I). Fetal heart rate was unaffected (Table I).

Maternal and fetal blood gases. Maternal arterial P_{O_2}, P_{CO_2}, and pH did not change in response to nicardipine treatment (Table II). In contrast, fetal blood gas values were significantly affected. Fetal arterial pH declined from control values of 7.331 ± 0.009 to significantly lower levels after infusion (Table II). The maximal decline in fetal arterial pH was observed at 90 minutes with a mean value of 7.268 ± 0.016 ($p < 0.01$) (Table II).

Fetal arterial P_{CO_2} increased dramatically after the start of nicardipine treatment with significant differences observed at 60 and 90 minutes. Peak values, which were observed at 90 minutes, were significantly higher than control levels (45.2 ± 4.2 versus 35.5 ± 2.4 mm Hg, respectively) ($p < 0.01$) (Table II). The most striking alterations were observed in fetal arterial P_{O_2}. Values were significantly depressed throughout the period of nicardipine treatment. The maximum decline in fetal arterial P_{O_2} was observed at 60 minutes (16.8 ± 2.2 versus 24.7 ± 2.1 mm Hg for control) ($p < 0.01$) (Table II).

Maternal and fetal plasma nicardipine concentrations. Maternal and fetal plasma nicardipine concentrations are illustrated in Fig. 2. Nicardipine was readily detectable in fetal blood within 15 minutes after a maternal intravenous bolus injection of 500 μg. During the constant 1-hour infusion nicardipine concentrations in fetal blood rose nearly in parallel with levels in maternal blood. Peak maternal plasma nicardipine concentrations were observed at 45 and 60 minutes with peak values ranging from 175 to 865 ng/ml. Fetal concentrations peaked at 60 minutes with maximum values ranging from 7 to 35 ng/ml. Fetal plasma concentrations did not exceed 6% of maternal levels. When the intravenous infusion was discontinued maternal and fetal plasma nicardipine concentrations declined in a

rapid and parallel fashion and reached 35% of maximum levels within 45 minutes. Nicardipine was essentially undetectable in the amniotic fluid.

Comment

The results of the present study confirm and extend our previous observations on the effects of tocolytic doses of calcium entry blockers in the pregnant rhesus monkey.[9] The two studies together clearly demonstrate that although calcium entry blockers of the dihydropyridine type are effective tocolytic agents in primates, other inherent problems may limit their clinical utility. Specifically, deleterious alterations are observed in fetal blood gas parameters. Fetal arterial pH, P_{CO_2}, and P_{O_2} were all markedly altered in response to maternal nicardipine administration. The question remains, what are the factor(s) responsible for these changes?

We had previously speculated that because of the high degree of binding to plasma protein, calcium entry blockers of the dihydropyridine type would likely be limited in their extent of placental transfer.[9] To our knowledge, the present study is the first to quantitate fetal levels of nicardipine in the primate fetus after maternal administration. Although variable, fetal levels never exceeded 6% of maternal plasma concentrations. Transplacental passage of nicardipine therefore is minimal in the primate. The low circulating levels of nicardipine in the fetus did not have a significant effect on fetal cardiovascular dynamics. Fetal heart rate remained unchanged and there was a minimal tendency for fetal mean arterial pressure to decline (Table I).

In the present study it is difficult to assess the effect of the decline in maternal mean arterial pressure on placental perfusion, since blood flow measurements were not made. Mean arterial pressure declined only 10% from control values after nicardipine administration. Golichowski et al.[12] used a 30-minute constant infusion of nifedipine to pregnant sheep and observed a 12% fall in maternal blood pressure at a dose of 10 μg/kg/min. Veille et al.[13] administered a bolus injection of nifedipine (20 to 40 μg/kg) to pregnant pygmy goats and observed a 10% decline in maternal arterial pressure and a transient fall in uterine blood flow in some animals. In a similar study of pregnant ewes, Harake et al.[8] measured a 21% drop in uterine blood flow in response to nifedipine (10 μg/kg/min). Since uterine vascular resistance was unchanged, the drop in uterine blood flow was attributed solely to the maternal hypotensive effect of the calcium entry blocker. Fetal arterial pH and oxygen saturation also decreased. Other studies in pregnant rabbits given nicardipine (36 μg/kg/min) also demonstrated an appreciable decline in uterine blood flow that was related to maternal hypotension.[14] It was reported that maternal nicardipine administration can alter the distribution of fetal cardiac output without changing total cardiac output.[8, 15] Increases in brain and myocardial blood flow but not in umbilical blood flow were observed in chronically catheterized fetal sheep.[8, 15]

Although species differences may exist, one would expect similar mechanisms to operate in the rhesus monkey. However, in a previous study we observed that with continuous infusions of nifedipine (3 μg/kg/min) (without a preinfusion bolus) no change in maternal arterial pressure occurred.[9] Despite this fact, the decline in fetal arterial pH and P_{O_2} that was observed was similar in magnitude to the changes observed with nicardipine administration in the present study. Parisi et al.[7] found that nicardipine (20 μg/kg/min) administered to hypertensive ewes in doses sufficient to render them normotensive also resulted in fetal hypoxemia and acidosis. In some instances, fetal death occurred. The study by Parisi et al.[7] also demonstrated that angiotensin II vasoconstriction was reversed by nicardipine in the systemic and uterine vessels but not in the placenta. It seems logical then to conclude that the compromise in fetal oxygenation observed in most studies is due in part to a decrease in uterine blood flow in response to maternal hypotension but that other factors (for example, redistribution of uteroplacental blood flow or shunting producing a maternal-fetal perfusion imbalance) likely play an important role.

Earlier studies in which nifedipine was used to treat premature labor in women documented a marked tocolytic effect with no apparent compromise of neonatal outcome.[4, 5] The present study and other observations in experimental animals cast a shadow of concern over the use of these agents for routine clinical tocolysis.[7-9] A major problem in comparing clinical and experimental studies, apart from obvious species differences, is the dosage and route of administration. Animals in the present study received a 500 μg intravenous bolus followed by a continuous infusion of 6 μg/kg/min for 1 hour. This equals a total dose of approximately 2.7 mg per rhesus monkey (average weight = 6 kg) or 0.45 mg/kg. The common dosage of calcium entry blocker used in clinical trials is 20 to 30 mg administered orally or sublingually every 6 to 8 hours or 0.40 to 0.60 mg/kg. Therefore the amount of nicardipine used in this study is in the range recommended for human use. Although the majority of clinical studies use oral administration instead of intravenous injection, maternal uptake of calcium entry blockers is quite rapid. Ramesch and Sommer[16] demonstrated detectable levels of nifedipine in the maternal circulation 6 minutes after oral administration, with maximum concentrations reached after 1 hour.

It is noteworthy that in studies in pregnant sheep, the fetal-to-maternal plasma concentration ratios were 0.3 to 0.4 during nifedipine infusions.[8] In the monkey

the nicardipine ratio was <0.1. On the basis of placental type, one would predict the monkey placenta to be more permeable, but earlier studies have suggested the important effect of protein binding on placental transfer of calcium entry blockers.[2] Nifedipine and nicardipine are both dihydropyridine derivatives but nicardipine also has a potent phosphodiesterase inhibitory action.[17] Although nicardipine has a slower onset of action it is more potent than nifedipine in relaxing isolated strips of human pregnant myometrium.[17]

In conclusion, the present study demonstrates that nicardipine crosses the placenta in measurable quantities and may adversely affect fetal oxygenation by a variety of mechanisms. Calcium entry blockers are potentially useful uterine tocolytic agents, but more detailed studies of their hemodynamic and metabolic side effects in the fetus are required before this potential can be fully and safely realized.

We wish to acknowledge the expert technical assistance of Michael J. Cook. In addition, we wish to thank Dr. Milan Henzl at Syntex Research for his invaluable assistance.

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Temporal changes in uterine activity and prostaglandin response to RU486 in rhesus macaques in late gestation

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Progesterone withdrawal as a mechanism of parturition in primates is controversial because maternal, fetal, and amniotic fluid progesterone concentrations do not decrease before parturition. We therefore studied the effects of RU486 on uterine activity and amniotic fluid prostaglandins in four rhesus macaques implanted with amniotic fluid and maternal vascular catheters and with fetal electrocardiogram and uterine electromyogram electrodes at 119 to 124 days' gestation (term = 168 days). Uterine electromyogram, intra-amniotic pressure (hourly contraction area, mm Hg · sec/hr), and fetal electrocardiogram were monitored continuously. After a stabilization period (6 to 9 days) RU486 was administered orally (20 mg/kg/day) at 1000 hours for 3 days. Uterine activity increased from basal levels (<4000 mm Hg · sec/hr) 8 hours after the first dose of RU486, reaching levels of 12,000 mm Hg · sec/hr. A sustained increase in uterine activity (13,000 to 30,000 mm Hg · sec/hr) was observed for 48 hours before cesarean section with little or no cervical effacement or dilatation. Increases in amniotic fluid prostaglandin $F_{2\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and 11-deoxy-13,14-dihydro-15-keto-11β, 16ε-cycloprostaglandin E_2 occurred 40 hours after the onset of increased uterine activity. In contrast, amniotic fluid prostaglandins in the control animals delivering at term (n = 4) increased 24 to 48 hours before significant increases in uterine activity occurred. Control animals but not those given RU486 demonstrated a progressive nocturnal peak in uterine activity before delivery. Progesterone receptor blockade stimulates intense preterm uterine activity but not the orderly sequence of changes in prostaglandins and cervical status observed during normal parturition. (Am J Obstet Gynecol 1987;157:1487-95.)

Key words: RU486, prostaglandins, uterine activity, progesterone receptors

The inhibitory effect of progesterone on the pregnant myometrium is well documented in lower mammals, and in those species progesterone withdrawal is a necessary step in the sequence of events leading to spontaneous labor and successful delivery of a viable fetus.[1] However, progesterone withdrawal, as a mechanism for parturition in primates, is controversial because of sustained antepartum levels of this hormone in maternal and fetal blood and in amniotic fluid.[2, 3]

The availability of synthetic progestin antagonists provides a useful tool to investigate the effects of functional progesterone withdrawal on hormonal control of the initiation of parturition. RU486 is a potent synthetic 19-norsteroid that interacts with the progesterone receptor and antagonizes the action of this hormone in all species studied so far. Preliminary investigations in monkeys and human beings indicate that the administration of RU486 effectively induces abortion in early pregnancy[4, 5] and is somewhat less effective for induction of delivery during late gestation in nonhuman primates.[4]

The nature of the biologic effects of RU486 on the pregnant primate uterus and cervix have not previously been described. We therefore administered RU486 to chronically catheterized rhesus macaques during late pregnancy and report here its effects on uterine contractility, cervical ripening, and the associated changes in amniotic fluid prostaglandins and maternal plasma steroid hormone concentrations.

Material and methods

Animals. Timed pregnant rhesus macaques *(Macaca mulatta)* were obtained from the breeding colony at the Oregon Regional Primate Research Center. At approximately day 110 of pregnancy, the animals were conditioned to a jacket and tether system.[6] The animals were maintained on their normal diet of 25% protein Purina Monkey Chow and fruit throughout the conditioning and experimental periods.

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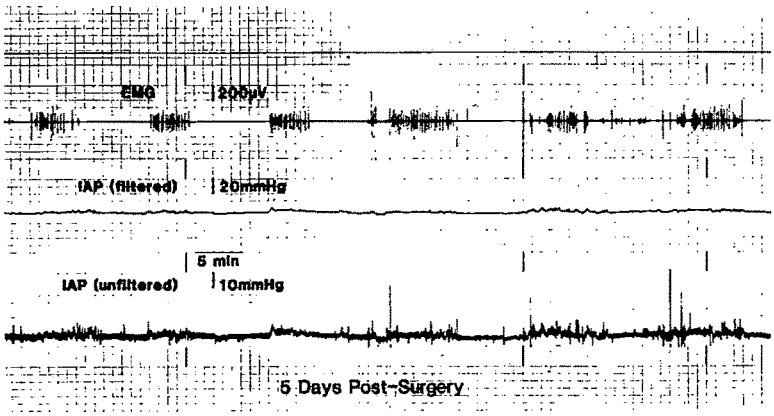


Fig. 1. Section of a representative recording from the quiescent uterus of a rhesus macaque at 5 days after operation showing electromyogram *(EMG)* and intra-amniotic pressure *(IAP)* during a 1-hour period. The uteri of all the animals in this study returned to this state of quiescence after the initial postoperative increase in activity and remained quiescent until treatment with RU486 or spontaneous delivery.

Nine animals were used in this study. Maternal femoral arterial and venous catheters, two open-ended intra-amniotic pressure catheters, two sets of myometrial bipolar electromyographic electrodes, and fetal electrocardiographic electrodes were implanted in each animal in the surgical suite at the research center according to the method of Novy et al.[7] All animals received terbutaline sulfate (1 mg/150 ml 5% dextrose in water over 3 to 5 hours, b.i.d.) for 24 to 48 hours after operation, to control surgically induced uterine activity, and indomethacin (50 mg orally b.i.d.) for 48 hours after operation, as an analgesic. Operation took place on days 119 to 124 of gestation for RU486-treated animals and on days 116 to 140 for control animals (term = 168 days). Six to nine days after operation the five experimental animals received RU486 at a dose of 20 mg/kg/day for 3 days at 10:00 AM each day. The fetuses were delivered by cesarean section 72 hours after the initiation of RU486 treatment. The four control animals received no treatment except for vehicle control in the diet and postoperative care, and they delivered spontaneously on days 156 to 167 after being monitored in the tether assembly for 24 to 45 days.

Uterine activity and cervical status. Uterine activity (electromyographic and intra-amniotic pressure) was recorded continuously on a Hewlett-Packard eight-channel recorder beginning immediately after operation until cesarean section or spontaneous delivery and was simultaneously monitored with a TEK-31 programmable calculator, as described by Ducsay et al.[8] Uterine contractility is expressed as the area under the contraction curve per hour (hourly contraction area in mm Hg · sec/hr). Electromyographic activity was not quantified but is described qualitatively relative to amniotic fluid pressure.

The cervix was palpated vaginally immediately after operation, before each dose of RU486 (time 0, +24, and +48 hours), and before cesarean section (+72 hours). Consistency, effacement, and dilatation of the cervix were recorded at each examination.

Collection and storage of plasma and amniotic fluid samples. Maternal plasma and amniotic fluid samples were collected every 3 days and then every 12 hours (9:00 AM and 9:00 PM) for 72 hours before and after RU486 administration in experimental animals. Plasma and amniotic fluid samples were collected every 3 days for control animals until parturition was imminent and every 12 hours at 9:00 AM and 9:00 PM thereafter. Plasma and amniotic fluid samples were frozen after centrifugation and stored at −20° C. All samples were assayed within 1 month after collection.

Radioimmunoassays. Maternal plasma was used to measure progesterone, estradiol and estrone levels according to the method of Resko et al.,[9, 10] and cortisol according to the method of Krey et al.,[11] with antisera obtained from Radioassay Systems Laboratories, Inc., Carson, Calif. The cross-reactivity of pure RU486 in the present progesterone assay system was <0.01%.

Amniotic fluid samples were also used to measure concentrations of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), and 11-deoxy-13,14-dihydro-15-keto-11β,16ϵ-cycloprostaglandin E_2 (PGEM-II). Authentic $PGF_{2\alpha}$, PGFM, 6-keto-$PGF_{1\alpha}$ and PGEM-II were obtained from The Upjohn Company, Kalamazoo, Mich. [5,6,8,9,11,12,14,15]-^{3}H-$PGF_{2\alpha}$ (195

Ci/mmol), 13,14-dihydro-15-keto-[5,6,8,9,11,12,14]-^{3}H-$PGF_{2\alpha}$ (157 Ci/mmol), and [5,8,9,11,12,14,15]-^{3}H-6-keto-$PGF_{1\alpha}$ (157 Ci/mmol) were purchased from Dupont (New England Nuclear Research Products, Boston, Mass.), and 13,14-dihydro-15-keto-[5,6,8,11,12,14]-^{3}H-PGE_2 was purchased from the Amersham Corp., Arlington Heights, Ill. Anti-$PGF_{2\alpha}$–bovine serum albumin serum was obtained from Dr. Leon Speroff. Anti–6-keto-$PGF_{1\alpha}$–bovine serum albumin serum was obtained from Professor G. C. Liggins, Auckland, New Zealand. Rabbit anti-PGFM and anti-PGEM-II were obtained from Dr. F. Fitzpatrick of The Upjohn Company, Kalamazoo, Mich. Validation of the PGFM and PGEM-II assays was published previously.[12] Intra-assay and interassay coefficients of variation were 7.2% and 15.8%, respectively, for the PGFM assay and 7.3% and 15.7%, respectively, for the PGEM-II assay.

$PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were measured in separate aliquots of 0.3 ml and 0.5 ml. After the amniotic fluid was divided into aliquots, approximately 1600 dpm of ^{3}H-$PGF_{2\alpha}$ or ^{3}H-6-keto-$PGF_{1\alpha}$ in 0.1 ml of assay buffer (0.1 mol/L phosphate buffer, 0.9% [wt/vol] sodium chloride, 0.1% [wt/vol] sodium azide, 0.1% [wt/vol] gelatin, pH 7.4), was added to monitor procedural losses and then acidified (pH 3.5 to 4.0, $PGF_{2\alpha}$; pH 0.3, 6-keto-$PGF_{1\alpha}$) by adding 30 μl of 1.0 mol/L formic acid. The prostaglandins were extracted with 5 ml of freshly distilled diethyl ether and centrifuged at 1200 × g at 4° C. The diethyl ether was transferred to glass 12 by 75 mm tubes and evaporated under nitrogen. The residue was redissolved in 0.35 ml of distilled absolute ethanol, and 0.2 ml of the extract was transferred to a separate 12 by 75 mm polypropylene tube for radioimmunoassay. A 0.1 ml aliquot of the same extract was counted to determine procedural losses.

After evaporating the ethanol, 0.2 ml of anti-$PGF_{2\alpha}$–bovine serum albumin or anti–6-keto-$PGF_{1\alpha}$–bovine serum albumin serum was added to the appropriate radioimmunoassay tubes and preincubated for 20 minutes at room temperature before the addition of either ^{3}H-$PGF_{2\alpha}$ or ^{3}H-6-keto-$PGF_{1\alpha}$ (20,000 dpm). A standard curve and amniotic fluid pools were run in each assay. After overnight incubation at 4° C, free and antibody-bound prostaglandins were separated with 1.0 ml of dextran-coated charcoal suspension (300 mg charcoal, 30 mg dextran T-70 in 100 ml assay buffer). The bound tritiated prostaglandins were subjected to liquid scintillation counting after the supernatants were decanted.

The linear range of the logit bound/unbound versus log dose standard curves was 10 to 1000 pg for $PGF_{1\alpha}$ and 7.8 to 1000 pg for 6-keto-$PGF_{1\alpha}$ and the sensitivity was 10 pg and 7.8 pg, respectively. Buffer blanks in each of the prostaglandin radioimmunoassays were below the sensitivity of the assay and were not subtracted.

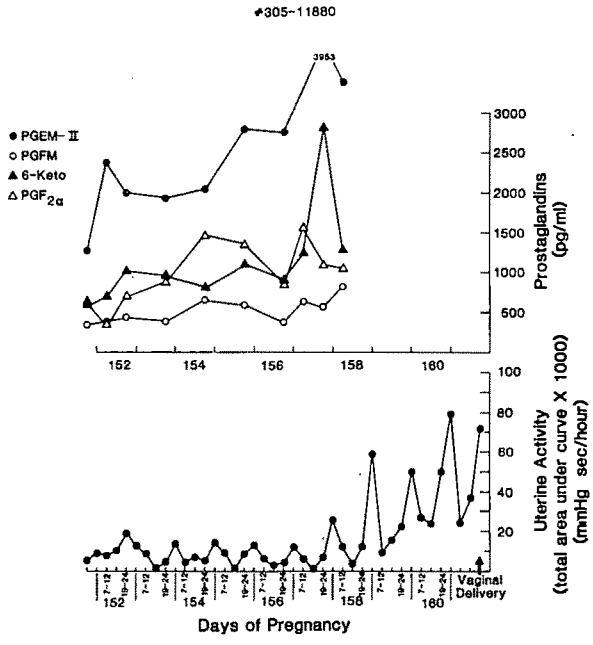


Fig. 2. Uterine activity and amniotic fluid prostaglandin concentrations in a control animal. Each point on the uterine activity scale represents the combined area under the intra-amniotic pressure contraction curve for 6-hour periods: 1:00 to 6:00 AM, 7:00 AM to 12 noon, 1:00 to 6:00 PM, and 7:00 PM to 12 midnight. The contraction area for any given hour never exceeded 20,000 mm Hg · sec/hr. The last amniotic fluid sample obtained was on day 158 of pregnancy. An increase in prostaglandin concentrations is evident before the increase in uterine activity.

Accuracy of the $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ radioimmunoassays were assessed by recovery experiments in which known amounts of each prostaglandin, ranging from 78 to 2500 pg, were added to individual 1.0 ml aliquots of amniotic fluid obtained from pregnant rhesus macaques. Linear regression analysis of the concentration measured *(y)* versus the prostaglandin concentrations added *(x)* yielded the following equations and correlation coefficients: $PGF_{2\alpha}$, $y = 1.21x + 7.9$, $r = 0.995$ (n = 4); 6-keto-$PGF_{1\alpha}$, $y = 0.94x + 57.9$, $r = 0.945$ (n = 2). Parallelism in each radioimmunoassay was tested in serially diluted aliquots of rhesus amniotic fluid spiked with the appropriate prostaglandin. In each instance, the resulting curve was parallel to the corresponding standard curve. Intra-assay and interassay coefficients of variation were 8.3% and 10.7%, respectively, for the $PGF_{2\alpha}$ radioimmunoassays and 7.7% and 13.4%, respectively, for the 6-keto-$PGF_{1\alpha}$ radioimmunoassays. PGE_2, $PGF_{1\alpha}$, PGFM, and 6-keto-$PGF_{1\alpha}$ cross reacted 1.0%, 2.91%, 0.05%, and 0.64%, respectively, with the $PGF_{2\alpha}$ antibody. PGE_2, $PGF_{2\alpha}$, and PGFM cross reacted 11.9%, 3.4%, and <0.01%, respectively, with the 6-keto-$PGF_{1\alpha}$ antibody. Cross-

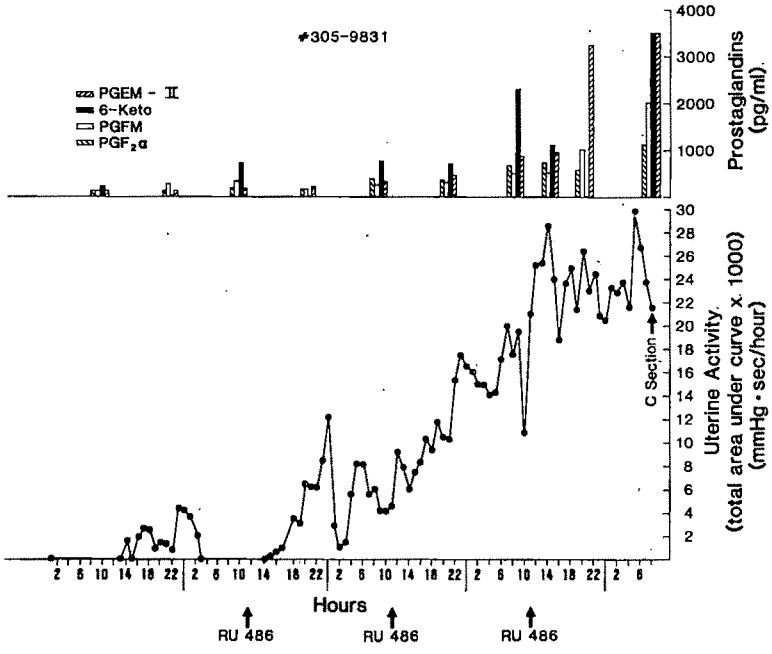


Fig. 3. Uterine activity and amniotic fluid prostaglandin concentrations in a rhesus macaque at 130 to 133 days' gestation treated with RU486 in three daily doses of 20 mg/kg/day *(arrows)*. Each point on the uterine activity graph represents the contraction area for 1 hour. Time of day is indicated on the abscissa.

reaction was defined as $(x/y) \times 100$, where x is the mass of either $PGF_{1\alpha}$ or 6-keto-$PGF_{1\alpha}$ and y is the mass of heterologous prostaglandin at which the binding of the appropriate radioligand is reduced by 50%.

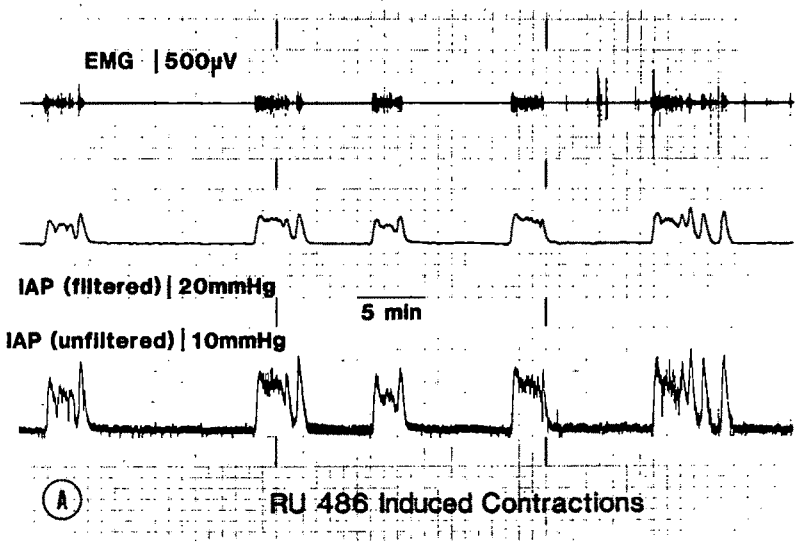
Statistics. Significant differences were determined by completely randomized one-way analysis of variance, followed by Duncan's multiple range test.

Results

Uterine activity. After an initial postsurgical increase, uterine activity (electromyogram and intra-amniotic pressure) returned to baseline levels within 48 hours after operation and remained at that level during the control period as shown 5 days after operation in Fig. 1. Electromyogram bursts occurred at a frequency of three to five per hour with minimal responses in intra-amniotic pressure. When increases in intra-amniotic pressure did occur in response to electromyogram activity, they were usually seen around midnight. Total activity did not exceed 4000 mm Hg · sec/hr and was usually <1000 mm Hg · sec/hr. All animals treated with RU486 exhibited this degree of uterine quiescence before treatment. Control animals that delivered spontaneously at term maintained a low level of uterine activity until approximately 1 week before parturition, when the nocturnal periods of activity gradually increased in intensity. These animals demonstrated a progressive increase in uterine activity beginning 2 to 3 days before vaginal delivery, with peaks in activity occurring around midnight and in the early morning hours (Fig. 2). During the 2 or 3 days before delivery, nighttime peak activity ranged between 10,000 and 30,000 mm Hg · sec/hr with lower levels of uterine activity observed during the day. In all cases, control animals demonstrated a definite diurnal variation in uterine activity, as illustrated in Fig. 2.

In contrast, animals treated with RU486 demonstrated an increase in uterine activity that reached 2000 to 12,000 mm Hg · sec/hr within 8 hours after the first dose of RU486. A transient decrease in activity occurred during the early morning hours after the first dose, which was typical for all animals, but uterine activity increased again before the second dose (Fig. 3). After the second dose of RU486, uterine activity rose progressively and reached a plateau of continuous activity at 13,000 to 30,000 mm Hg · sec/hr, which was sustained until cesarean section was performed at +72 hours. No diurnal pattern was noted.

The individual contractions occurring in animals treated with RU486 were quantitatively and qualitatively different from the contractions seen in control animals. RU486-treated animals had long-duration (3 to 7 minutes), low-frequency (five per hour), and high-amplitude (40 to 50 mm Hg) contractions that were always associated with long bursts of electromyogram activity (Fig. 4, *A*). In contrast, control animals in the final stages of labor exhibited high-frequency (about one per minute), short-duration contractions



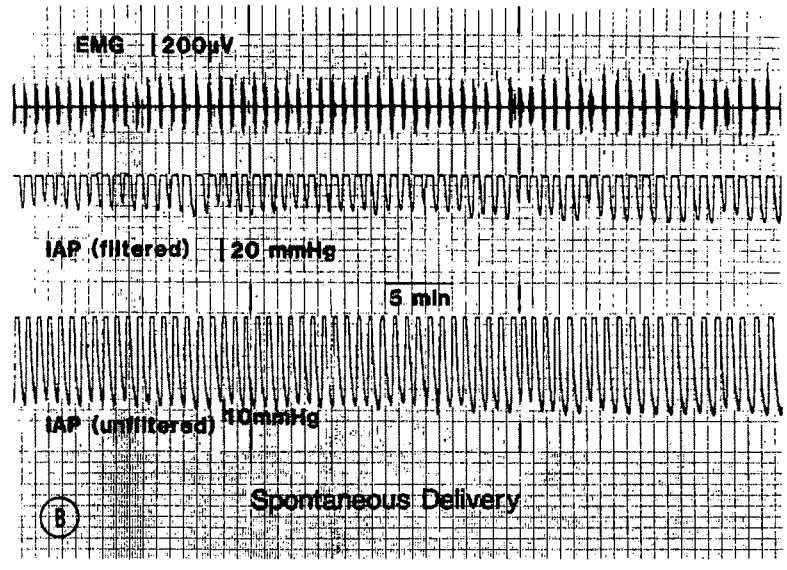


Fig. 4. Uterine activity recordings for a period of 1 hour from two different rhesus macaques demonstrating electromyogram *(EMG)* activity and corresponding intra-amniotic pressure *(IAP)* increases. *(A)* 48 hours after the beginning of RU486 treatment; *(B)* just before spontaneous delivery. The sensitivity of the electromyogram signal is 500 μV/cm in Fig. 4, *A*, and is different from that (200 μV/cm) in Figs. 1 and 4, *B*.

with an amplitude >50 mm Hg (Fig. 4, *B*). The sinusoidal waveform during active labor in control animals is similar to the nocturnal contractions seen during the last 2 to 3 days before delivery, but with lower frequency and amplitude, and were always associated with bursts of myometrial electromyogram activity.

Cervical status. The cervices of animals Nos. 9269 and 9831 changed from firm to soft within 24 hours after the first dose of RU486; a 30% effacement occurred in monkey No. 9269 at 24 hours but did not change thereafter. A 50% effacement and a 5 mm dilatation occurred in the cervix of monkey No. 9831 after 72 hours of RU486 treatment. The other two animals (Nos. 11795 and 10378) showed no change in any of the cervical parameters measured despite intense uterine activity for 72 hours. Control animals typically demonstrated progressive cervical effacement during the week before spontaneous vaginal delivery. These changes were accelerated and associated with cervical dilatation as the prepartum uterine activity intensified.

Mode of delivery and fetal status. Control animals

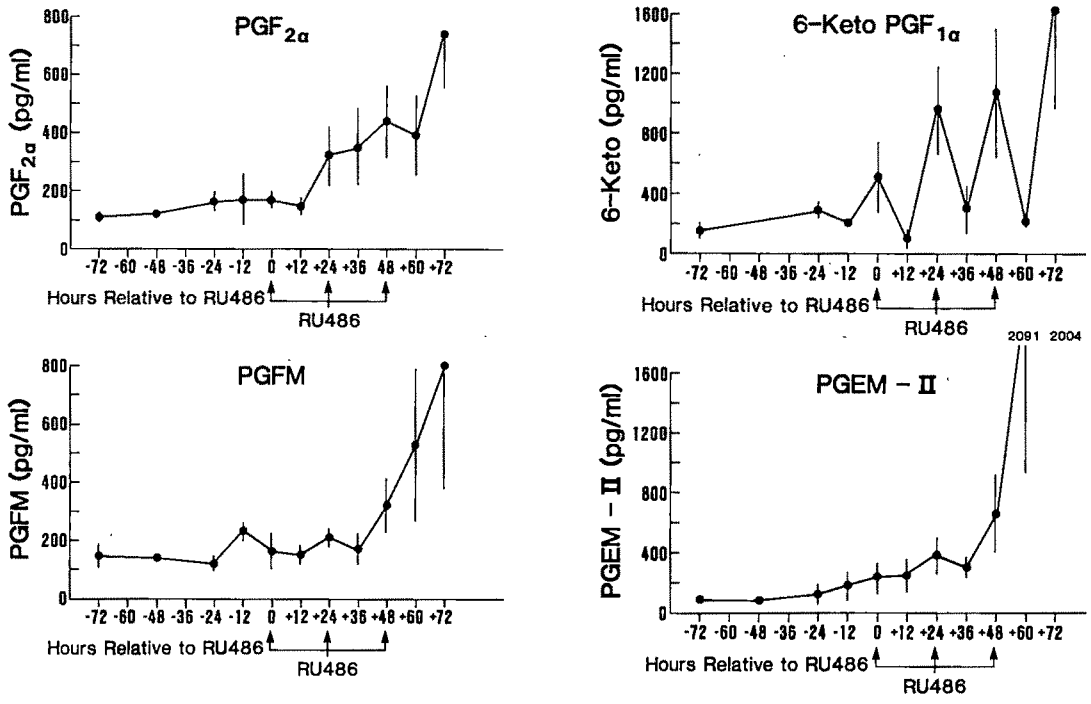


Fig. 5. Mean (±SEM) concentration of individual prostaglandins in rhesus macaques (n = 4) for 72 hours before and after RU486 administration.

were monitored for 25 to 45 days before delivery at or near term. Animal No. 11768 was delivered by cesarean section because of fetal malpresentation while in spontaneous labor with the cervix fully dilated. All other control animals delivered vaginally.

One of five RU486-treated animals underwent cesarean section 48 hours after treatment because of fetal death. At autopsy, no apparent cause of the fetal death was found, and the data for this animal were not included in the results. Repetitive and prolonged decelerations of the fetal heart rate were common in RU486-treated animals, but in spite of this, four of the five treated animals delivered live fetuses by cesarean section 72 hours after being treated with RU4886. Cesarean section was necessary because of absent cervical effacement and dilatation.

Prostaglandins. There was no apparent change in amniotic fluid prostaglandin concentrations in animals treated with RU486 until 40 hours after uterine activity began to increase or 48 hours after treatment began (Fig. 5). The first apparent change was seen in 6-keto-$PGF_{1\alpha}$ levels, which demonstrated a periodic fluctuation every 12 hours after RU486 treatment. The peak concentration of 6-keto-$PGF_{1\alpha}$ at +72 hours was significantly higher ($p < 0.05$) than previous values, except for determinations at time 0, +24 hours, and +48 hours relative to RU486 administration. The increase in amniotic fluid PGFM and PGEM-II after +48 hours did not reach statistical significance because of wide variation among animals in the concentrations of PGFM at +60 and +72 hours. Lack of an adequate amniotic fluid sample for the PGEM-II assay limited the number of data points at +60 and +72 hours. However, in the two animals for which samples were available, a nearly tenfold elevation in amniotic fluid PGEM-II was observed after RU486 treatment (300 to 400 pg/ml at +36 hours versus 2000 to 3000 pg/ml at +60 and +72 hours). The concentration of $PGF_{2\alpha}$ at +72 hours was significantly elevated ($p < 0.05$) over all values recorded earlier with the single exception of the concentration at +48 hours.

In contrast, in animals delivering spontaneously at term, amniotic fluid prostaglandins began to increase 24 to 48 hours before the rise in spontaneous uterine activity; PGEM-II increased before the other prostaglandins measured (Fig. 3). Amniotic fluid samples could not be obtained in some control animals for 24 to 72 hours before delivery because of low amniotic fluid volume or rupture of the fetal membranes. An elevation in all amniotic fluid prostaglandins was observed during the week before parturition with a significant increase in PGEM-II 3 to 6 days before birth (Table I).

Steroid hormones. The concentration of estrone and cortisol in maternal plasma did not change significantly during the experimental period for RU486-treated animals. Maternal plasma progesterone concentrations were significantly increased ($p < 0.05$) at +72 hours

Table I. Mean (±SEM) concentrations of $PGF_{2\alpha}$, PGFM, 6-keto-$PGF_{1\alpha}$, and PGEM-II at various time points before spontaneous labor and delivery in four rhesus macaques

Day before parturition	*$PGF_{1\alpha}$ (pg/ml)*	*PGFM (pg/ml)*	*6-keto-$PGF_{1\alpha}$ (pg/ml)*	*PGEM-II (pg/ml)*
1-2	815 ± 180	498 ± 61	1493 ± 457	1773 ± 670*†
3-4	1091 ± 291	803 ± 271	1597 ± 587	3134 ± 906†
5-6	867 ± 273	459 ± 64	1393 ± 400	2766 ± 15†
7	712 ± 261	492 ± 114	908 ± 399	1318 ± 526*†
14	375 ± 60	266 ± 35	841 ± 378	1160 ± 463*†
21	260 ± 84	220 ± 40	259 ± 88	448 ± 339*
28	324 ± 224	191 ± 81	428 ± 331	486 ± 453*

Values with different superscripts (*, †) are significant at $p < 0.05$.

Table II. Mean (±SEM) concentrations of progesterone, estrone, estradiol, and cortisol in maternal plasma of four rhesus macaques treated with RU486

Time relative to RU486 (hr)	*Progesterone (ng/ml)*	*Estrone (pg/ml)*	*Cortisol (ng/ml)*	*Estradiol (pg/ml)*
−72	2.5 ± 0.3	264.0 ± 6.0	219.8 ± 36.0	262 ± 58
−48	3.6 ± 0.5	277.0 ± 16.2	352.6 ± 50.5	364 ± 98
−24	2.9 ± 0.4	373.3 ± 41.7	198.9 ± 36.6	382 ± 43
0	3.1 ± 0.5	331.6 ± 44.6	222.9 ± 43.3	354 ± 34
+24	3.1 ± 0.4	358.8 ± 44.7	203.0 ± 29.7	433 ± 72
+48	4.0 ± 0.5	395.8 ± 74.3	217.1 ± 23.4	463 ± 78
+72	7.1 ± 1.8	523.3 ± 86.7	256.0 ± 89.7	578 ± 98

relative to RU486 treatment. Maternal plasma estradiol concentration at +72 hours was significantly different ($p < 0.05$) only from the level at −72 hours (Table II).

Comment

This study demonstrates for the first time that progesterone receptor blockade in a primate species stimulates intense uterine activity but not the orderly sequence of changes in uterine activity profiles, amniotic fluid prostaglandins, and cervical ripening that is observed during normal parturition.

Rhesus macaques delivering spontaneously at or near term demonstrate a progressive increase in uterine activity beginning 2 to 3 days before vaginal delivery with peaks in activity occurring near midnight. Similar peaks in uterine activity have been described in rhesus macaques delivering prematurely at 130 to 155 days' gestation.[8] Spontaneous prepartum uterine contractions in rhesus macaques are characteristically of high frequency, of short duration, and of high amplitude during periods of peak activity. They are associated with progressive cervical effacement and dilatation. Three very important components are missing after RU486 administration, in contrast to the spontaneous onset of labor: (1) a diurnal pattern in myometrial activity, (2) the transition from long electromyogram events to shorter electrical bursts with sinusoidal contractions, and (3) increased cervical effacement and dilatation.

The RU486-treated animals demonstrated an increase in uterine activity within 8 hours after oral administration followed by a plateau of intense uterine activity with no diurnal variation (Fig. 3). A comparison of Figs. 1 and 4 indicates that the electrical events associated with RU486-induced activity are similar in duration and amplitude to electromyogram events in the quiescent uterus, but an increase in the number of individual potentials within electromyogram bursts after RU486 treatment is associated with a striking increase in intra-amniotic fluid pressure. This may indicate greater coupling between myometrial smooth muscle cells, but it is not clear why the RU486-induced contractions are different from the contractions observed during spontaneous labor.

In spite of sustained high levels of uterine activity for 3 days, the cervices of treated animals showed little effacement or dilatation, and cesarean section was necessary to deliver viable fetuses. It appears that blockade of the progesterone receptors alone is not sufficient to effect cervical compliance. The late response in amniotic fluid prostaglandins with absent cervical changes in RU486-treated monkeys (as compared with the prelabor increase in prostaglandins and cervical softening in spontaneous parturition) suggests that prostaglandins mediate cervical ripening and that an orderly sequence is necessary for effective labor. This is supported by data demonstrating that prostaglandin gels applied to the cervix enhance Bishop scores and improve the outcome of induced labor.[13] Relaxin, which is implicated in cervical softening, may also be deficient.

In a single previous study of RU486 administration

in nonhuman primates, cynomolgus monkeys received RU486 (20 mg/kg/day) for 3 days at various stages of gestation.[4] Abortion occurred in two of three animals treated at 30 to 40 days' gestation. Six monkeys received RU486 between days 130 and 155 of gestation; four of the six animals were primed with 10 mg/day of estradiol benzoate. Two monkeys were reportedly unaffected by the treatment, but uterine activity was not monitored. One monkey delivered a dead fetus at 171 days' gestation. The remaining three animals delivered dead fetuses within 4 to 10 days after the onset of RU486 treatment. Obstructed labor was noted in all three monkeys and cesarean section was performed in two. Our experience suggests that the obstetric difficulties described by Germain et al.[4] in cynomolgus monkeys treated with RU486 were secondary to cervical dystocia. It is of interest that by the seventh week of human gestation and more clearly thereafter, successful interruption of pregnancy by RU486 diminishes primarily because of incomplete expulsion of the conceptus.[5]

Although our study adds to the evidence that progesterone is involved in the suppression of myometrial electrical and mechanical activity during pregnancy, the mechanisms of the suppressive effect of progesterone on myometrial contractile response or on the liberation of arachidonic acid metabolites have yet to be clarified. Progesterone has a dual action on arachidonic acid metabolism to the extent that it promotes the storage of prostaglandin precursors in decidua and membranes while stabilizing lysosomes containing acylhydrolases.[14] Classical steroid receptor-acceptor action may be linked to myometrial gap junction formation[15] but not to prostaglandin production by fetal membranes since estrogen and progesterone receptors are absent in human term amnion and chorion.[16] Progesterone may act in a similar way to glucocorticoids in stimulating a 40,000 molecular weight protein (lipomodulin) that inhibits phospholipase activity.[17]

In addition, this study shows that the normal physiologic antepartum increase in prostaglandins is not solely dependent on the loss of inhibition by progesterone. This finding suggests active stimulation of prostaglandin production before parturition by other stimuli, e.g., estrogens or fetal factors. However, an endogenous stimulus to prostaglandin synthesis in the primate uterus has yet to be demonstrated conclusively. An important role for oxytocin in the triggering mechanism is suggested by rising decidual oxytocin receptors at term and by in vitro stimulation of prostaglandin production by human decidua.[18] Casey et al.[19] found that a substance in human fetal urine causes a dose-dependent increase in PGE_2 synthesis by amnion cells in monolayer culture. The demonstration that relaxin and prolactin are formed in human decidua suggests a potential regulatory role of these hormones in prostaglandin production.

Some progestomimetic activity of RU486 has been described in humans and rhesus macaques, but paradoxically this activity is lost in the presence of physiologic progesterone concentrations.[20] RU486 also possesses potent antiglucocorticoid activity as well as antiprogesterone activity. Dexamethasone is known to ablate diurnal rhythmicity and reduce uterine activity in rhesus macaques.[8] The effect seen by Ducsay et al.[8] was related to fetal adrenal suppression and reduced estrogen biosynthesis or to stimulation of lipomodulin synthesis. Theoretically the antiglucocorticoid effect of RU486 could synergize with the antiprogesterone effect in stimulating uterine activity when administered to monkeys. The reported effects of RU486 on peripheral levels of adrenocorticotropic hormone and cortisol are dose dependent and variable.[21] We observed no effect of RU486 on the cortisol and estrogen levels in maternal plasma until 72 hours after treatment (Table II). Moreover, the potential role of RU486 in stimulating uterine activity by antiglucocorticoid mechanisms is minimal on the basis of the tenfold to fiftyfold higher potency of RU486 as an antiprogestin.[22]

The availability of RU486 provides new opportunities to investigate the endocrine and paracrine mechanisms controlling the onset of parturient uterine activity and in the successful management of induced labor in late pregnancy. At the present time neither the safe dose, the optimal route of administration, nor the duration of RU486 therapy (needed to achieve a minimal risk-to-benefit ratio) has been established for labor induction. Future work may establish a basis for using RU486 as an adjunct to prostaglandins and/or relaxin for the safe induction of labor in the third trimester of pregnancy.

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Cerebral oxidative metabolism in fetal sheep with multiple-dose ethanol infusion

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Cerebral oxidative metabolism and cotylendonary blood flow were measured in 10 unanesthetized fetal sheep (127 to 132 days' gestation) during a control period, after the first, third, and fourth infusions of four doses of 0.5 gm of ethanol per kilogram of maternal body weight infused into the ewe during 5 hours, and 24 hours after ethanol infusion. Preductal arterial and sagittal vein blood samples were analyzed for oxygen content, blood gases, pH, and ethanol. Cerebral and cotylendonary blood flow were measured with a radioactive microsphere technique. Fetal blood gases and pH changed little with the ethanol infusions, although PaO_2 and oxygen content decreased 24 hours after ethanol infusion. Cotylendonary blood flow, which was decreased after the third and fourth ethanol infusions, did not account for these delayed hypoxemic changes. Similarly, cerebral oxidative metabolism was decreased when measured after each of the ethanol infusions, with no dose response or tolerance evident. This noted fall in fetal cerebral oxidative metabolism appears to be a direct depressant effect that was maximal at rather low fetal ethanol levels, which, if prolonged, might well affect cerebral growth and development. Recovery of cerebral metabolic function appeared complete by 24 hours. However, relative fetal hypoxemia was evident at this time, the mechanism of which remains to be determined. (AM J OBSTET GYNECOL 1987;157:1496-502.)

Key words: Ethanol, cerebral oxidative metabolism

Prominent among the defects noted in infants exposed to excessive maternal alcohol intake are abnormalities in brain growth and structural development[1] and dysfunction of the central nervous system,[2, 3] with an apparent dose-response relationship for these effects. Abnormalities in brain growth and development are also noted in the rat embryo exposed to ethanol, with mechanisms involving decreased protein synthesis and cellular hypoplasia.[4, 5]

We have previously shown that a short-term infusion of moderate amounts of ethanol to pregnant ewes (1.0 $gm \cdot kg^{-1}$ over a 1-hour period) results in alterations in the fetal state with the emergence of an intermediate-voltage electrocortical state and a decrease in fetal cerebral oxidative metabolism that is still evident 1 hour after ethanol infusion.[6] We hypothesized that if prolonged, this change in fetal state and decrease in cerebral oxidative metabolism might well affect fetal brain development and provide a mechanism whereby chronic alcohol intake contributes to growth anomalies and dysfunction of the central nervous system in infants exposed to alcohol in utero. It is not known, however, whether the decrease in fetal cerebral oxidative metabolism is a direct effect of the ethanol or caused by the change in fetal sleep state noted, since we have recently demonstrated a significant fall in cerebral oxygen consumption in the fetal lamb with a change from a low-voltage electrocortical state.[7] The ethanol dose–cerebral oxidative metabolism response relationship and time course for recovery also remain to be determined.

Therefore the purpose of the present study was to examine the effect of a multiple-dose maternal ethanol infusion on fetal cerebral oxygen consumption to determine if a dose-response relationship exists along with the time course for recovery and also to determine the extent to which behavioral state changes are involved in these effects. Because fetal hypoxia has also been proposed as a mechanism for ethanol-induced growth anomalies,[8] cotyledonary blood flow was measured in relation to noted fetal blood gas changes.

Material and methods

Surgical procedures. Studies were performed on 10 fetal sheep of mixed breed between 127 and 132 days' gestation (term 147 days). Fetuses were prepared for study at least 3 days before initiation of all experiments. The ewes were anesthetized with 1.5 to 2.0% halothane

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Table I. Measurements of fetal cardiovasculature and arterial metabolites from experimental periods

		Ethanol infusions			
	Control	*First dose*	*Third dose*	*Fourth dose*	*24 hr*
Ethanol (mg/ml)	ND	0.81 ± 0.05*	1.84 ± 0.12*	2.37 ± 0.16*	0.08 ± 0.08
pH	7.32 ± 0.01	7.32 ± 0.01	7.33 ± 0.01	7.33 ± 0.01	7.32 ± 0.01
P_{O_2} (mm Hg)	22.6 ± 0.9	24.1 ± 0.8	24.1 ± 1.1	24.1 ± 0.8	20.8 ± 0.9†
P_{CO_2} (mm Hg)	49.1 ± 0.8	49.2 ± 0.8	48.1 ± 0.6	47.6 ± 1.2	50.7 ± 0.4†
Hemaglobin (gm/dl)	9.5 ± 0.5	9.5 ± 0.5	9.9 ± 0.4	9.7 ± 0.5	9.4 ± 0.4
Mean blood pressure (mm Hg)	46 ± 4	46 ± 4	44 ± 4	44 ± 4	43 ± 3
FHR (bpm)	171 ± 5	174 ± 7	163 ± 6	158 ± 5	158 ± 6

ND = Not detected.

Values are means ± SEM; n = 10 except for 24 hours where n = 9. Mean blood pressure and FHR values were averaged for the time during each blood flow determination and 15 minutes thereafter.

*$p < 0.01$.

†$p < 0.05$.

and 50% oxygen to 50% nitrous oxide, and the uterus was exposed and opened through a lower abdominal midline incision. Polyvinyl catheters were placed in the brachiocephalic trunk via a fetal forelimb artery for sampling preductal arterial blood and in the femoral artery via a fetal hindlimb artery for sampling postductal arterial blood. A catheter was placed in the inferior vena cava via a fetal pedal vein, and the sagittal sinus was instrumented and sampled as previously reported.[9] Tracheal and amniotic fluid catheters were also placed as previously described.[9] Electrodes of stainless steel, Teflon-coated wire (Cooner, Chatsworth, California) were implanted biparietally on the dura for fetal electroencephalographic recordings, through the orbital ridge of the zygomatic bone of each eye for electroocular recordings, and over the sternum for fetal heart rate (FHR) recordings. A reference electrode was placed in the loose connective tissue overlying the occipital bone of the skull. The catheters, which contained heparinized saline solution, and wires were exteriorized through the flank of the ewe, and the abdomen was closed in layers. Polyvinyl catheters were then placed in the maternal femoral artery and vein. At the time of surgery, 1 gm of dihydrostreptomycin and 800,000 U of penicillin G were injected intramuscularly into the ewe, and after the uterus was closed, 1,000,000 U of penicillin G were injected into both the fetal hindlimb vein and the amniotic cavity. This antibiotic was continued daily for 2 to 3 days. After surgery, animals were maintained in individual cages suitable for continuous monitoring, with food and water available.

Physiologic measurements. Each fetal lamb was studied during a control period, after the first, third, and fourth of four multiple doses of ethanol infused into the ewe over 5 hours, and 24 hours after ethanol infusion. Strain-gauge manometers (Statham Model P-23 ID, Gould, Oxnard, California) and a strip-chart recorder (Model 78D, Grass Instrument, Quincy, Massachusetts) were used to record pressures in the trachea, amniotic fluid, and the brachiocephalic artery. Mean arterial blood pressure was calculated as diastolic plus 0.4 of systolic minus diastolic pressure. Electrical signals were processed with a common mode rejection preamplifier (Model 7 P511J, Grass Instrument). FHR was measured by means of a cardiotachometer (Model 7P44B). Fetal electrocortical and electroocular potentials were displayed directly on the chart recorder after passing through a passive band-pass filter, 0.3 to 30 Hz, on the preamplifier.

Continuous recordings of electrocortical, electroocular, tracheal pressure, amniotic fluid pressure, fetal arterial blood pressure, and FHR were begun 24 hours before ethanol infusion. Approximately 1 hour before ethanol infusion and during a period of high-voltage electrocortical with electroocular activity absent, radionuclide-labeled microspheres were injected into the fetal inferior vena cava for determination of regional blood flow distribution. This procedure was followed immediately by two paired blood samples, 5 minutes apart, which were drawn simultaneously from the brachiocephalic artery and the sagittal vein. Four doses of 0.5 gm ethanol per kilogram of maternal body weight were then infused into the maternal vena cava of each ewe over a 5-hour period. Each dose, as a 40% v/v solution of ethanol (Alcool, Liquor Control Board of Ontario), was administered by an infusion pump over 30 minutes, with a 1-hour interval between each dose to allow extravascular distribution of ethanol in the maternal and fetal compartments before each successive dose.[10] After the first, third, and fourth ethanol doses and again 24 hours after ethanol infusion, microsphere injections were again performed, followed by a paired blood sampling from the brachiocephalic

Table II. Cerebral metabolic measurements

Measurements	Control	Ethanol infusions			24 hr
		First dose	Third dose	Fourth dose	
C_{AO_2} (mmol/L)	2.9 ± 0.2	2.9 ± 0.2	2.9 ± 0.2	2.7 ± 0.2	2.2 ± 0.1 (9)*
Blood flow (ml · 100 gm^{-1} · min^{-1})	157 ± 15	114 ± 11†	115 ± 15‡	124 ± 7	190 ± 19 (7)
Oxygen delivery (μmol · 100 gm^{-1} · min^{-1})	441 ± 35	321 ± 20†	317 ± 35†	320 ± 29‡	393 ± 17 (7)
Oxygen consumption (μmol · 100 gm^{-1} · min^{-1})	127 ± 9 (8)	98 ± 7 (8)*	98 ± 8 (8)‡	98 ± 9 (7)†	115 ± 12 (6)
Oxygen fractional extraction	0.31 ± 0.01 (8)	0.32 ± 0.03 (8)	0.35 ± 0.03 (8)	0.34 ± 0.03 (7)	0.29 ± 0.02 (6)
Arteriovenous ethanol difference (mg/ml)	ND	0.02 ± 0.02 (8)	0.02 ± 0.03 (8)	0.08 ± 0.05 (8)	0.01 ± 0.01 (6)

ND = Not detected; C_{AO_2} = arterial oxygen concentration.
Values are means ± SEM; n = 10 except where otherwise indicated; significance based on paired data.
*$p < 0.01$.
†$p < 0.02$.
‡$p < 0.05$.

artery and sagittal vein. Fetal monitoring was continued throughout this time. Although the fetal state was not controlled for, in 25 of the 30 measurements after ethanol infusion and in eight of the nine measurements at 24 hours, the fetus was either in the high-voltage or an intermediate-voltage electrocortical state.

Each blood sample was analyzed for oxygen content, pH, P_{O_2}, P_{CO_2}, and ethanol. Results were averaged for the two control period samples to obtain a mean value. With each paired blood sample, approximately 1 ml of arterial blood and 1 ml of venous blood were withdrawn.

Oxygen content was determined in triplicate by means of a Lex-O_2-Con (Lexington Instruments, Waltham, Massachusetts) blood gas analyzer. Blood pH, P_{O_2}, and P_{CO_2} were analyzed on an ABL-3 blood gas analyzer with temperature corrected to 39° C (Radiometer, Copenhagen, Denmark). Blood ethanol concentration was measured by gas-liquid chromatography with the use of headspace analysis as previously described.[10]

Regional blood flow was measured with 15 μm diameter microspheres (New England Nuclear, Boston, Massachusetts, and 3M, New Brighton, Minnesota) labeled with one of five different radioisotopes (Cerium 141, chromium 51, strontium 85, niobium 95, or scandium 46) by means of methods previously published.[9] Reference samples were withdrawn from both the brachiocephalic and femoral arteries at a rate of 2.40 ml/min via a Harvard infusion withdrawal pump (Harvard Apparatus, Dover, Massachusetts). At the completion of each microsphere injection and subsequent blood sampling, estimated fetal blood loss was replaced with maternal blood.

On completion of the studies, the ewe and fetus were killed, and an autopsy was performed on the fetus to validate catheter placement. The fetal brain was weighed and dissected into the following regions: right and left cerebral hemispheres, subcortical structures (thalamus, epithalamus, hypothalamus, corpus striatum, and midbrain), cerebellum, pons, and medulla. The fetal cotyledons were dissected free of the myometrial tissue and fetal membranes were trimmed. All tissues were weighed separately and analyzed for radioactivity (Compugamma Model 1282, LkB Wallac Oy, Turku, Finland). Blood flow to these tissues was then calculated as previously described by Makowski et al.[11]

Data analysis. Results obtained from the 10 animals for control, ethanol infusion, and postethanol infusion periods are presented as grouped means ± SEM. One fetal lamb, with normal blood gases and pH during the control period, PaO_2 18.7 and pH 7.35, developed progressive metabolic acidosis after the fourth ethanol dose, with fetal demise occurring approximately 18 hours after ethanol infusion. In some experiments catheter failure prevented the completion of all studies in each fetus. Cerebral oxygen consumption values were calculated as the product of arteriovenous difference and cerebral blood flow. Cerebral blood flow only included flow to the cerebral hemispheres, since this is the region drained primarily by the sagittal sinus. Cerebral oxygen delivery was calculated as the product of arterial oxygen content and hemispheric blood flow. Cerebral fractional extraction of oxygen was calculated from the arteriovenous difference divided by arterial oxygen content. Electrocortical activity was assessed by visual analysis into periods of high voltage (100 to 200 μV), low-voltage (<50 μV), and intermediate-voltage electrocortical (50 to 100 μV). The data reported in Ta-

Table III. Changes in blood flow to specific regions of the fetal brain

Regions	*Control*	*Ethanol infusions*			*24 hr*
		First dose	*Third dose*	*Fourth dose*	
Cerebral hemisphere	157 ± 15	114 ± 11*	115 ± 15†	124 ± 7	190 ± 19
Subcortex	204 ± 15	162 ± 18†	192 ± 30	190 ± 18	307 ± 28‡
Cerebellum	197 ± 16	172 ± 17	198 ± 21	219 ± 28	263 ± 24†
Pons	217 ± 19	188 ± 23	240 ± 40	229 ± 29	332 ± 35†
Medulla	245 ± 24	214 ± 23	272 ± 36	290 ± 30	336 ± 27*

Values are means (ml · 100 gm^{-1} · min^{-1}) ± SEM; n = 10 except for 24 hours where n = 7; significance based on paired data.
*$p < 0.02$.
†$p < 0.05$.
‡$p < 0.01$.

bles I to III were examined by analysis of variance for repeated measurements followed by the Newman-Keuls test if a significant F ratio was obtained ($p < 0.05$). Fetal breathing movements, electrooocular activity, and electrocortical activity, which were monitored continuously both before and after ethanol infusion, are reported separately,[12] as is the pharmacokinetics and pharmacodynamics of ethanol in the maternal fetal unit.[13]

Results

During the experiments ewes did not exhibit unusual agitation; however, during the multiple-dose ethanol infusions, the sheep appeared to have impairment of balance and spent a greater part of the time lying down. They returned to regular activity within 6 hours after ethanol infusion with no apparent ill effect.

Fetal cardiovascular and arterial metabolic measurements from the experimental periods are shown in Table I. The 5-hour multiple-dose ethanol infusion resulted in a stepwise increase in ethanol concentrations of fetal arterial blood to a maximum of 2.37 mg/ml after the fourth ethanol infusion. Although fetal arterial blood gases changed little during ethanol infusions, 24 hours after infusion, P_{O_2} significantly decreased ($p < 0.05$), whereas P_{CO_2} significantly increased ($p < 0.05$). No significant differences were noted in fetal hemoglobin concentration or in mean arterial blood pressure or FHR measured at the time of blood flow determinations.

Maternal arterial pH declined progressively during multiple-dose ethanol infusions from a control value of 7.44 ± 0.01 to 7.38 ± 0.01 after the fourth ethanol infusion ($p < 0.01$). Maternal arterial pH increased significantly 24 hours after infusion, 7.47 ± 0.01, when compared with control values ($p < 0.05$). These pH changes were largely metabolic in nature, since the base excess also showed a progressive decline from 0.6 ± 0.9 to −4.8 ± 0.7 mmol/L after the fourth ethanol infusion ($p < 0.01$) and then increased to 1.8 ± 0.9 mmol/L, although not significantly, when measured 24 hours after infusion. Maternal arterial P_{O_2} and P_{CO_2} changed little throughout the study when compared with control values, 101.4 ± 2.8 and 36.1 ± 0.5 mm Hg, respectively.

Cerebral oxygen delivery. Fetal arterial oxygen content, although unchanged during ethanol infusions, also significantly decreased when measured 24 hours after ethanol infusion ($p < 0.01$; Table II). Blood flow to the cerebral hemispheres was significantly decreased after both the first and third doses of ethanol ($p < 0.02$ and $p < 0.05$, respectively), and although still decreased after the fourth dose, it was not significant (Table II). The decrease in cerebral hemispheric blood flow with little change in arterial oxygen content resulted in a significant decrease in cerebral oxygen delivery after each of the ethanol infusions where measured (Table II).

Cerebral oxygen consumption. Cerebral oxygen consumption was 127 ± 9 μmol · 100 gm^{-1} · min^{-1} during the control period and decreased significantly where measured after each of the ethanol infusions (Table II), with a related fall in cerebral blood flow; the arteriovenous oxygen difference remaining little changed. Cerebral oxygen consumption returned to control values when measured 24 hours after ethanol infusion. The cerebral fractional extraction of oxygen, as a measure of the oxygen consumed as a fraction of that delivered, remained little changed throughout the study (Table II).

There was no measured consumption of ethanol by the brain, since the cerebral arteriovenous ethanol difference was negligible both with the infusions and 24 hours after infusion (Table II).

Regional blood flow. Control blood flows were determined during an episode of a high-voltage electrocortical state whereas subsequently electrocortical states were variable during blood flow determinations but again were usually of high or intermediate voltage.

Changes in blood flow to specific regions of the fetal brain are presented in Table III and Fig. 1. Regional

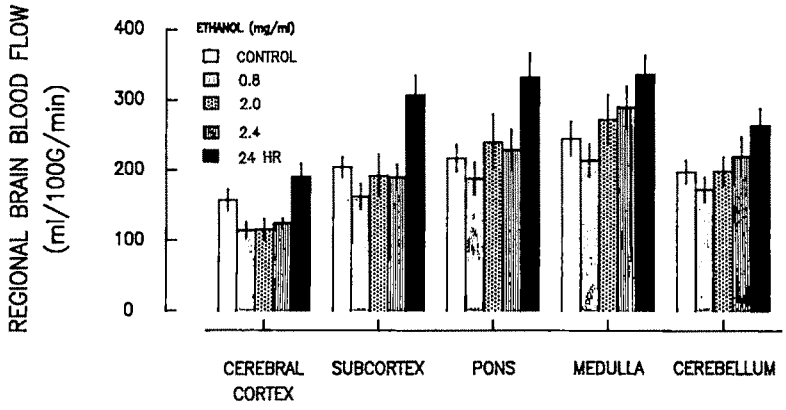


Fig. 1. Mean (± SEM) blood flows to the different brain regions during the control period, after the first, third, and fourth ethanol infusions, and 24 hours after ethanol infusion.

differences in the effect of ethanol on blood flow to the brain were evident, with flow to the cerebral hemispheres significantly decreased after both the first and third ethanol infusions (a 27% decrease), flow to the subcortical tissues significantly decreased after only the first ethanol infusion (a 20% decrease), and flow to the other brain tissues showing no significant change. Twenty-four hours after ethanol infusion, blood flow significantly increased in all regions of the brain except the cerebral hemispheres, with the increase most pronounced in the subcortex (a 50% increase) and the pons (a 53% increase).

Blood flow to the fetal cotyledons was determined during the control and ethanol infusion periods in seven of the study animals and 24 hours after ethanol infusion in five of the study animals. Cotyledonary blood flow was 193 ± 26 ml · kg^{-1} · min^{-1} during the control period and decreased significantly after both the third, 144 ± 23 ml · kg^{-1} · min^{-1} ($p < 0.01$) and the fourth, 136 ± 21 ml · kg^{-1} · min^{-1} ($p < 0.01$) ethanol infusions. Twenty-four hours after infusion, cotyledonary blood flow returned to control values, 178 ± 23 ml · kg^{-1} · min^{-1}.

Comment

The regimen of four doses of 0.5 gm ethanol per kilogram of maternal body weight administered over a 5-hour period, with each dose infused over 30-minutes, and a 1-hour interval between each successive dose, was selected in an attempt to mimic a binge-type drinking episode. This multiple-dose infusion resulted in a stepwise increase in peak fetal arterial blood ethanol concentration to 2.37 ± 0.16 mg/ml reached at the end of the fourth ethanol infusion, which is higher than that reported in other studies involving the fetal lamb.[14-17] In the present study, fetal arterial blood gases and pH changed little during the ethanol infusions; however, 24 hours later, arterial P_{O_2} significantly decreased, whereas arterial P_{CO_2} significantly increased. As noted, one fetal lamb developed progressive metabolic acidosis, with fetal death occurring approximately 18 hours after ethanol infusion. Most other studies investigating the effects of ethanol have also noted little change in fetal blood gases and pH during the ethanol infusion,[14-16] but all have involved infusions of, at most, out to 3 hours, with postinfusion measurements only out to 5 hours. The fetal blood gas changes noted here at 24 hours must involve either a decrease in oxygen delivery and carbon dioxide excretion, for example, those involving a decrease in uterine blood flow or placental exchange mechanisms or an increase in fetal oxidative metabolism and carbon dioxide production. However, these changes in fetal blood gas could not be accounted for by corresponding changes in maternal blood gas or a fall in fetal cotyledonary blood flow, which changed little from control values when measured at 24 hours.

Maternal arterial pH fell progressively during the multiple-dose ethanol infusion, which reflects a developing metabolic acidosis as indicated by the corresponding decline in the base excess. This finding has been reported in other studies in which there was lower maternal ethanol exposure[14, 15] and may represent lactic acidosis since the redox changes associated with the oxidation of ethanol result in a shift of pyruvate to lactate.[18]

Fetal cerebral uptake of oxygen decreased 22% from the control value when measured after the first maternal ethanol infusion and remained similarly depressed when measured after the third and fourth infusions despite the stepwise increase in fetal arterial ethanol levels. Thus a dose-response relationship was not evident, with cerebral oxidative metabolism maximally depressed after the first ethanol infusion. Studies in adult humans[19] support the theory that dose responsiveness may also depend on the level of consciousness, since

mild ethanol intoxication is reported to cause little change in cerebral oxidative metabolism, whereas more severe intoxication with stupor is associated with depressed cerebral oxidative metabolism. Hemmingsen and Barry[20] have also noted a significant decrease in cerebral oxygen consumption with severe ethanol intoxication in acute adult rat studies.

In a previous study during which control measurements were made in the low-voltage electrocortical state and ewes were subsequently given a 1-hour infusion of ethanol at 1.0 gm · kg^{-1} over a 1-hour period, we noted a 35% decrease in the cerebral oxidative metabolism but with an associated change to a high- or intermediate-voltage electrocortical state in most animals.[6] Because we have also reported a significant fall in cerebral oxidative metabolism in the fetal lamb with a change from a low-voltage electrocortical state to a high-voltage electrocortical state,[7] the reported decrease in cerebral oxidative metabolism may have been, at least in part, an indirect effect caused by a change in the fetal sleep state. In the present study all control metabolic measurements were made during an episode of high-voltage electrocortical potential in which rapid eye movements were absent, whereas after each of the ethanol infusions, the fetal lambs were usually either still in a high-voltage electrocortical state or in a voltage state in between that of high and low voltage. Although the significance of this intermediate-voltage state is unknown, the decrease in cerebral oxidative metabolism noted in the present study suggests a more direct effect of ethanol on cerebral metabolic function and is in keeping with abnormal brain growth and development involving decreased protein and deoxyribonucleic acid synthesis reported with ethanol exposure in other animal studies.[4, 5] The lack of a dose-response relationship noted here indicates that the direct effects of ethanol on cerebral metabolic function are maximal at rather low fetal ethanol levels, which suggests that the degree of abnormal brain growth and development reported for both the animal model[4] and human infant[1] will be a function of the duration of maternal alcohol exposure rather than the intensity of acute exposure. In the present study cerebral oxygen consumption had returned to control levels by 24 hours at a time when fetal ethanol levels were again almost negligible, indicating no discernible continued direct effects of ethanol on cerebral metabolic function.

Blood flow to the cerebral hemispheres was decreased 27% after the first and third ethanol infusions and 21% after the fourth ethanol infusion. If this decrease were caused by a direct vasoconstrictive effect of ethanol, an increase in the extraction of oxygen might be expected, since cerebral arteriovenous oxygen difference has been found to vary inversely with changes in cerebral blood flow under conditions in which cerebral oxidative metabolism remains unchanged.[21] However, with the decrease in cerebral oxidative metabolism measured in this study, the significant fall in blood flow is consistent with reports of a close coupling of regional brain blood flow to metabolic rate.[22] A direct vasoconstrictive effect of ethanol is also possible and is supported by in vitro studies on canine cerebral arterioles where local application of ethanol results in a graded constriction that can be blocked by calcium antagonists.[23] When measured 24 hours after ethanol infusion, cerebral blood flow increased above control values, although not significantly, which is in keeping with the normal regulatory response to a drop in arterial oxygen content noted at this time; the fractional oxygen extraction thus remained relatively unchanged.

Regional differences in the effect of ethanol on brain blood flow were evident, with flow to the cerebral hemispheres significantly decreased after both the first and third ethanol infusions (a 27% decrease), flow to the subcortical tissues significantly decreased after only the first ethanol infusion (a 20% decrease), and flow to the other brain tissues showing no significant change. In our previous study,[6] the fall in brain blood flow with ethanol exposure was most pronounced in the subcortex and pons, findings similar to those we have reported with a change from a low- to a high-voltage electrocortical state[7] and that further support an alteration in fetal state as an indirect mechanism whereby ethanol alters cerebral oxidative metabolism. In the present study regional blood flow changes suggest that the direct depressant effect of ethanol on oxidative metabolism is largely confined to the cerebral hemispheres, given the close coupling of blood flow to metabolism in the brain. This finding supports a decrease in protein and deoxyribonucleic acid synthesis to this area of the brain with ethanol exposure during fetal development and is consistent with reports whereby abnormalities in brain growth and function are largely confined to cortical structures.[8] Regional blood flow changes at 24 hours are again in keeping with the normal fetal response to hypoxemia whereby the greatest increase occurs to the brainstem and deep cerebral structures.[24]

Blood flow to the fetal cotyledons was decreased during the time of ethanol exposure—25% after the third infusion and 30% after the fourth infusion—with a return to control values when measured 24 hours after ethanol infusion. The control values reported here are similar to those reported by other investigators using the chronically catheterized fetal lamb preparation;[25] however we could find no reports on the effects of ethanol exposure. Erskine and Ritchie [26] have shown that the impedance of the umbilical artery was unchanged in mothers exposed to alcohol between 34 and 36 week's gestation, suggesting little effect on vascular

resistance; however, maternal alcohol levels were much less than in the present study. Because mean arterial blood pressure changed little with the ethanol infusions, the fall in cotyledonary blood flow noted here must have involved an increase in umbilical vascular resistance, presuming the central venous pressure changed little. This decrease in cotyledonary blood flow would be expected to result in a decrease in fetal oxygen delivery, and thus oxygen levels and may have contributed to the small fall in arterial oxygen content noted after the fourth ethanol infusion. The lack of a more pronounced fall in fetal arterial oxygen levels might be explained by a decrease in overall fetal metabolic rate and thus oxygen consumption with the ethanol exposure.

Thus the decrease in fetal cerebral oxidative metabolism with maternal ethanol intoxication is both a direct depressant effect, possibly involving decreased protein and deoxyribonucleic acid synthesis, and an indirect effect involving a change in fetal state. The direct depressant effect is maximal at rather low fetal ethanol levels with no dose response evident and no evidence of tolerance developing at least during the 5 hours of ethanol exposure studied. Recovery of cerebral metabolic function appears complete by 24 hours. However, relative fetal hypoxemia is now evident, the cause of which remains to be determined, but may also contribute to the growth anomalies of infants exposed to alcohol in utero.

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Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant

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Antepartum fetal monitoring was initiated at 19 to 26 weeks' gestation in 15 pregnancies: six (five with systemic lupus erythematosus, one with circulating anticoagulant) with a complicated antepartum course (group 1); three, all systemic lupus erythematosus, with a normal antepartum course (group 2); and six normal control pregnancies (group 3). Group 1 all exhibited nonperiodic fetal heart rate decelerations, without the classical appearance of early, late, or variable decelerations, and four of the six had fetal bradycardia. In three group 1 cases, there was no active intervention because of early gestational age, and fetal death occurred at 23, 27, and 27 weeks, respectively. The other three patients in group 1 received betamethasone and were delivered by cesarean section at 28 to 30 weeks. There were no cases of respiratory distress syndrome or neonatal death. Five of the six infants in group 1 were small for gestational age. The nonperiodic fetal heart rate decelerations were absent in both groups 2 and 3 who all had normal fetal outcomes at term. The abnormal finding of women with nonperiodic fetal heart rate decelerations at 20 to 28 weeks may detect the fetus at risk for intrauterine death in pregnancies complicated by systemic lupus erythematosus or circulating anticoagulant. Continued surveillance, steroid induction of lung maturity, and delivery should be considered in these cases. (Am J Obstet Gynecol 1987;157:1503-10.)

Key words: Lupus, second trimester, nonperiodic decelerations, bradycardia, fetal death

Intrapartum electronic fetal monitoring has been shown to be reliable in the fetus who weighs ≤1500 gm.[1] Studies of antepartum fetal heart rate testing before 28 weeks' gestation have been done, but intervention in the form of delivery was often not performed because of fetal immaturity.[2-5] One parameter, fetal bradycardia during antepartum fetal heart rate testing, has been demonstrated to cause a high incidence of fetal distress in labor[6] as well as an increased incidence of antepartum fetal death and small for gestational age infants.[7, 8] The studies cited included fetuses at gestational ages of ≥32 weeks, with the exception of one patient who was evaluated at 27 weeks' gestation.[7] Current neonatal literature reports increasing survival rates of low birth weight newborn infants with a decreasing incidence of some handicaps.[9-12] These observations suggested that preterm delivery could be considered in some pregnancies with abnormal results of fetal heart rate testing.

This hypothesis can be tested with a specific high-risk group of mothers. We have previously shown that antibody to cardiolipin is a sensitive and specific indicator of fetal death and of fetal distress in pregnancies complicated by systemic lupus and/or circulating anticoagulant.[13] We now report our experience with the use of second-trimester (20 to 28 weeks) antepartum fetal heart rate testing to determine the timing of delivery in these pregnancies.

Material and methods

The duration of the study ranged from July 1980 through December 1986. Patients with systemic lupus erythematosus or circulating anticoagulant were selected for the study if they were referred for antepartum fetal heart rate testing before 26 weeks' gestation. The control patients were volunteers who agreed to undergo antepartum fetal heart rate testing starting before 26 weeks' gestation.

Fourteen patients (15 pregnancies) were evaluated by antepartum testing starting at <26 weeks' gestation. The patients were divided into three groups. Each patient's past obstetric history is detailed in Table I.

Group 1. All five patients (six pregnancies) in this

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Table I. Past obstetric history

Case No.	*Age (yr)*	*Gravidity*	*Parity*	*Abortion < 20 wk*	*Fetal death*
Group 1					
1	35	1	0		
2	26	4	0		3 (20+ wk)
3	19	2	0	1 (elective)	
4	29	1	0		
5	37	2	1		1 (27 wk)
6	36	2	1		1 (23 wk)
Group 2					
1	28	1	0		
2	33	2	0	1 (spontaneous)	
3	23	3	0	2 (spontaneous)	
Group 3					
1	29	4	2	1 (spontaneous)	
2	35	6*	1	3 (spontaneous)	
3	28	1	0		
4	28	2	1		
5	30	3	1		1 (36 wk)
6	28	2	0	1 (elective)	

*One ectopic pregnancy.

group had complicated systemic lupus erythematosus. Four of them had active systemic lupus erythematosus, defined either by abnormal serologic findings or clinical manifestations such as rash, arthritis, hypertension or renal disease. The fifth patient also had esophageal varices caused by portal vein thrombosis in infancy (Case No. 3). The fifth patient had circulating anticoagulant (Case No. 2). Four patients were taking prednisone and one was treated with aspirin alone (Case No. 5).

Group 2. Patients in this group had uncomplicated systemic lupus erythematosus and consisted of three patients with a verified diagnosis of systemic lupus erythematosus that was not active during the current pregnancy. None were taking medication.

Group 3. This group consisted of six normal volunteers with negative screening tests for systemic lupus erythematosus, circulating anticoagulant, and diabetes.

The antepartum testing scheme consisted of nonstress test as a primary screening test. A normal (reactive) nonstress test documents two or more accelerations of the fetal heart rate, each at least 15 beats/min above the baseline and associated with fetal movement, during a 20-minute monitoring period. If these criteria are not met in one of two successive 20-minute periods, the test is considered nonreactive and a contraction stress test is performed. Interpretation of the contraction stress test is made according to the criteria of Schifrin et al.[14]

Fetal bradycardia is defined as a decrease of ≥40 beats/min from the baseline heart rate or as a reduction to ≤90 beats/min lasting at least 60 seconds (Fig. 1). A nonperiodic deceleration is defined as a deceleration of at least 10 beats/min below the baseline without the classic appearance of early, late, or variable decelerations (Fig. 2). A biophysical profile was performed if the contraction stress test was contraindicated or if amniotic fluid volume was to be evaluated.[15]

All patients had routine prenatal care and underwent frequent antepartum fetal heart rate testing, ranging from once a week to almost daily. All patients in groups 1 and 2 were seen jointly by an obstetrician and a rheumatologist.

From July 1980 to August 1985, antibody to cardiolipin was determined as follows: Two hundred microliters of a solution of cardiolipin (Avanti Polar Lipids), 90 μg/ml of ethanol-chloroform, was placed in ml wells (Linbro), evaporated by air jet, and washed, after which 200 μl of a 1 : 10 dilution of test serum was added and incubated in duplicate specimens for 7 hours in a humid chamber at room temperature. The plates were then washed again. Antibody was measured by the enzyme-linked immunosorbent assay, with alkaline phosphatase–conjugated anti–polyvalent human immunoglobulin (Sigma Chemical Co., St. Louis, Mo.) incubated for 18 hours at 4° C, and developed with *p*-nitrophenyl phosphate in diethanolamine buffer. Color development was read at 405 nm in a Titertek multiscan photometer (Flow Laboratories, Meckenheim, West Germany). Replicate assays done the same day demonstrated <5% variability. All results were standardized to a single positive serum sample, with aliquots obtained in advance for this use and tested simultaneously. Arbitrarily, 100 units of reactivity corresponds to the reaction produced by 20 μl of this serum. The upper limit of normal was considered to be the mean ± 2 SEM. Values above 50 units were considered abnormal.[13]

After August 1985, the assay for antibody to car-

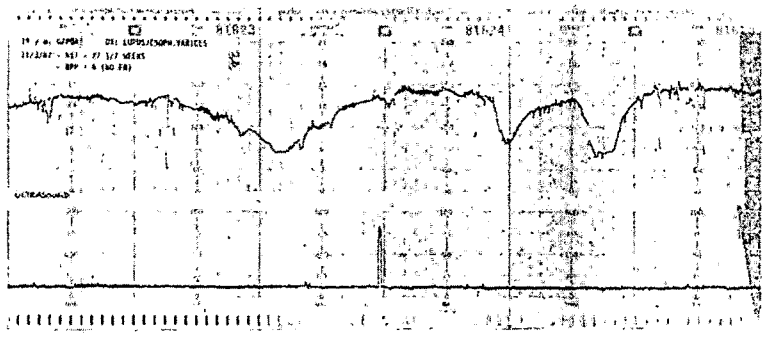


Fig. 1. Fetal bradycardia.

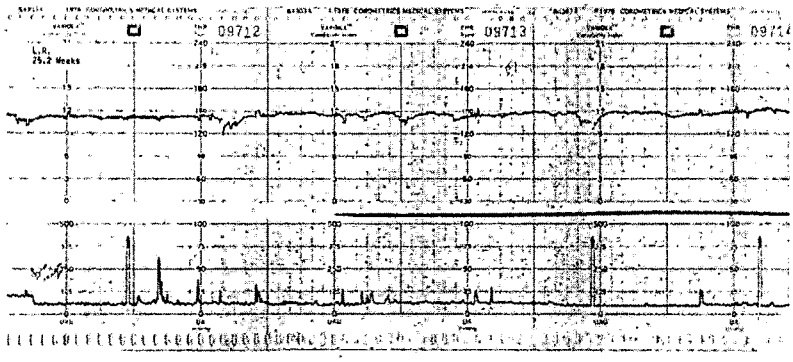


Fig. 2. Nonperiodic fetal heart rate decelerations.

diolipin was a modification of the method of Loizou et al.[16] and of the method described above, in which immunoglobulin-free fetal calf serum replaced gelatin and reagent quantities were reduced. Wells of Linbro microtiter plates were coated with 100 μl of cardiolipin, 12 μg/ml in ethanol. The plates were air dried, blocked with 10% fetal calf serum in phosphate-buffered saline. One hundred μl of serum from the patient diluted 1:100 in phosphate-buffered saline/10% fetal calf serum was added to the wells and incubated for 1 hour, then the plates were washed and developed with alkaline phosphatase–conjugated goat antihuman IgM or IgG in a standard enzyme-linked immunosorbent assay. Results were calculated as optical density at 405 nm of test well minus that of control well and normalized to a standard positive control run with each assay. Control wells containing no serum or those coated with irrelevant phospholipid uniformly gave background optical density readings of 0.18 to 0.20 in this assay. The upper limit of normal was defined as the average adjusted optical density for a normal value plus 3 SEM for 14 normal values (0.063 for IgM and 0.251 for IgG). When our previous method was compared with the new method, good correlation between the methods was obtained ($r^2 = 0.941$, $p < 0.01$).[17]

The routine clinical laboratory determined the activated partial thromboplastin time and the presence of lupus anticoagulant with citrated plasma, Actin FS activated partial thromboplastin time reagent (Dade [Becton-Dickinson], Grenoble, France), and an MFA Electra 700 Analyzer. Normal for this laboratory (mean + 4 SD) is <38 seconds. Abnormal results were confirmed at least once for each patient by mixing with normal plasma, but individual abnormal activated partial thromboplastin time results were not reconfirmed each time they occurred. The incidence of a positive lupus anticoagulant test in our obstetric population was not determined.

Amniotic fluid assessment defined oligohydramnios as the absence of at least one pocket of amniotic fluid measuring 2 by 2 cm. If only one pocket of fluid measuring 2 by 2 cm was present and no other fluid could be found, amniotic fluid volume was considered to be adequate but diminished.

Infant growth was assessed with the Brenner sea-level growth curve.[18]

In all six pregnancies from group 1, nonperiodic decelerations with or without bradycardia were noted after 20 weeks. In three cases (two with systemic lupus erythematosus and one with circulating anticoagulant) no intervention in the form of delivery was undertaken because of early gestational age and an estimated fetal

Table II. Pregnancy outcome

Case No.	*EGA at delivery or IUFD (wk)*	*Mode of delivery*	*Complications*	*Fetal outcome**
Group 1				
Intrauterine fetal death				
1	23	SVD	Hypertension Antibody to La Lupus nephritis	260 gm (SGA) Pericardial effusion
2	28.5	SVD	None	490 gm (SGA)
3	28	SVD	Esophageal varices	600 gm (SGA)
Live births				
4	28	C/S	Preeclampsia Thrombocytopenia	735 gm (SGA)
5	30.4	C/S	Fetal distress	650 gm (SGA)
6	30	C/S	Preeclampsia Thrombocytopenia	1145 gm (NEC)
Group 2				
1	40.4	C/S	Fetal distress	3315 gm
2	37.2	C/S	Thrombocytopenia	2200 gm (SGA)
3	41.5	C/S	Cephalopelvic disproportion	3960 gm
Group 3				
1	39.5	SVD		3910 gm
2	38.3	SVD		2840 gm
3	41.1	C/S	Cephalopelvic disproportion	4470 gm (LGA)
4	41.5	SVD	Meconium	4030 gm
5	38.4	SVD		3700 gm
6	41.4	SVD		2820 gm

EGA: Estimated gestational age; IUFD: intrauterine fetal death; SVD: spontaneous vaginal delivery; C/S: cesarean section; SGA: small for gestational age; LGA: large for gestational age; NEC: necrotizing enterocolitis.

*There were no congenital anomalies.

Table III. Antepartum fetal heart rate decelerations, number per test

	Group 1					
	Intrauterine fetal death		*Live births*			
	No. per test	*%*	*No. per test*	*%*	*Group 2 (No. per test)*	*Group 3 (No. per test)*
Nonperiodic decelerations*	33/37	89	25/48	52	0/57	0/59
Bradycardia†	8/37	22	1/48	4	0/57	0/59

*$p < 0.01$ (group 1 versus group 2 and group 3; intrauterine fetal death versus live births).

†$p < 0.05$ (intrauterine fetal death versus live births).

weight of <650 gm. This was done after consultation with the division of neonatology and an open discussion of the potential for survival of the fetus with the parents. Changes in medication were made, however, in two cases. In Case No. 1, prednisone was increased from 30 to 60 mg/day at 22 weeks. In Case No. 2, prednisone was gradually increased from 15 mg at 12 weeks to 30 mg from 14 to 23 weeks and up to 60 mg/day from 23 to 28 weeks. The medication was then decreased to 30 mg at 28 weeks and 20 mg at 29 weeks because of drug side effects. In the three other cases, all with systemic lupus erythematosus, betamethasone administration for induction of fetal lung maturity was instituted, and elective delivery by cesarean section was performed. Estimated fetal weight was ≥750 gm. Consultation with a neonatologist was obtained. The parents gave informed consent to the proposed plan for elective delivery.

In groups 2 and 3, pregnancy was allowed to continue in the absence of fetal and/or maternal compromise.

Results

The results are presented in Tables II and III.

Group 1. In three cases, where no intervention in the form of elective delivery was undertaken, fetal death occurred at 6, 1, and 4 days, respectively, after the last nonstress test. In the remaining three cases, betamethasone administration and delivery by cesarean

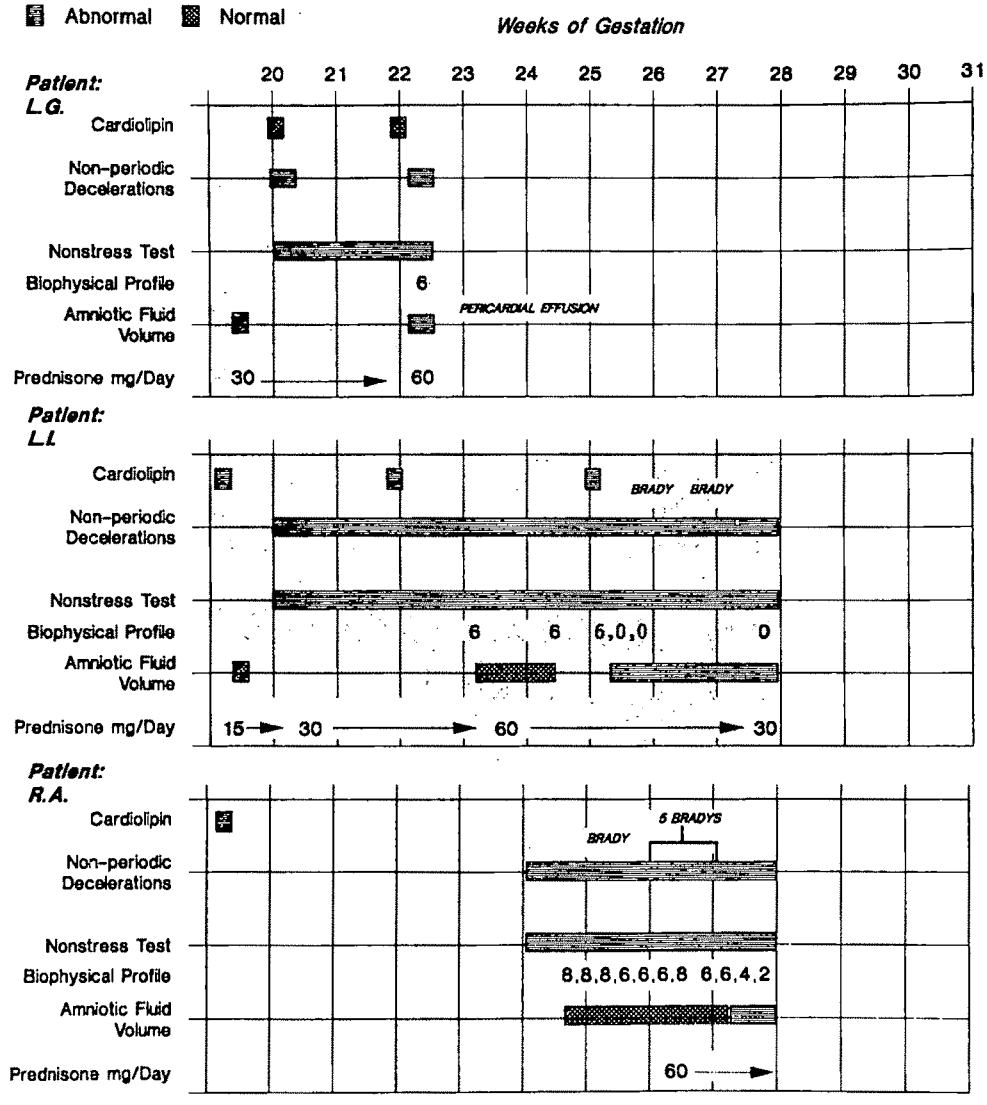


Fig. 3. Complicated lupus cases–fetal deaths.

section resulted in live births. All neonates had 1- and 5-minute Apgar scores >6.

Other indicators of fetal compromise, such as elevated antibody to cardiolipin, abnormal biophysical profile score, nonreactive nonstress tests, and decreased amniotic fluid volume, were less helpful in determining the timing of delivery (Figs. 3 and 4). The level of antibody to cardiolipin was abnormal in four out of six cases (Table IV). In all but two cases (fetal deaths, Cases Nos. 2 and 3), the biophysical profile score was ≥6 throughout the pregnancy. In the two cases of fetal death, the biophysical profile score became abnormal at 3 weeks and at 3 days, respectively, before the occurrence of fetal death. The percentage of nonreactive tests was significantly higher ($p < 0.01$) at 24 to 28 weeks' gestation in group 1 (88%) than in the other two groups (9% in group 2, 29% in group 3).

Fetal deaths. Fetal death occurred in three cases. In these three cases, sonographic evidence of oligohydramnios occurred, 2, 3, and 5 weeks, respectively, after detection of nonperiodic decelerations (Fig. 3). All three fetuses were small for gestational age. Pericardial effusion was documented on ultrasound 4 days before fetal death in Case No. 1. This patient had antibody to La antigen but not to Ro antigen. There was no evidence of congenital heart block. No unusual findings on the autopsy in the other two cases of fetal death were noted. Fetal bradycardia was the terminal pattern noted in two of the three cases of fetal death.

Live-born infants. This group consisted of three cases. Two of the three infants were small for gestational age. Oligohydramnios was noted 5 weeks after detection of nonperiodic decelerations in one case (Fig. 4) (Case No. 5). The other two cases had normal

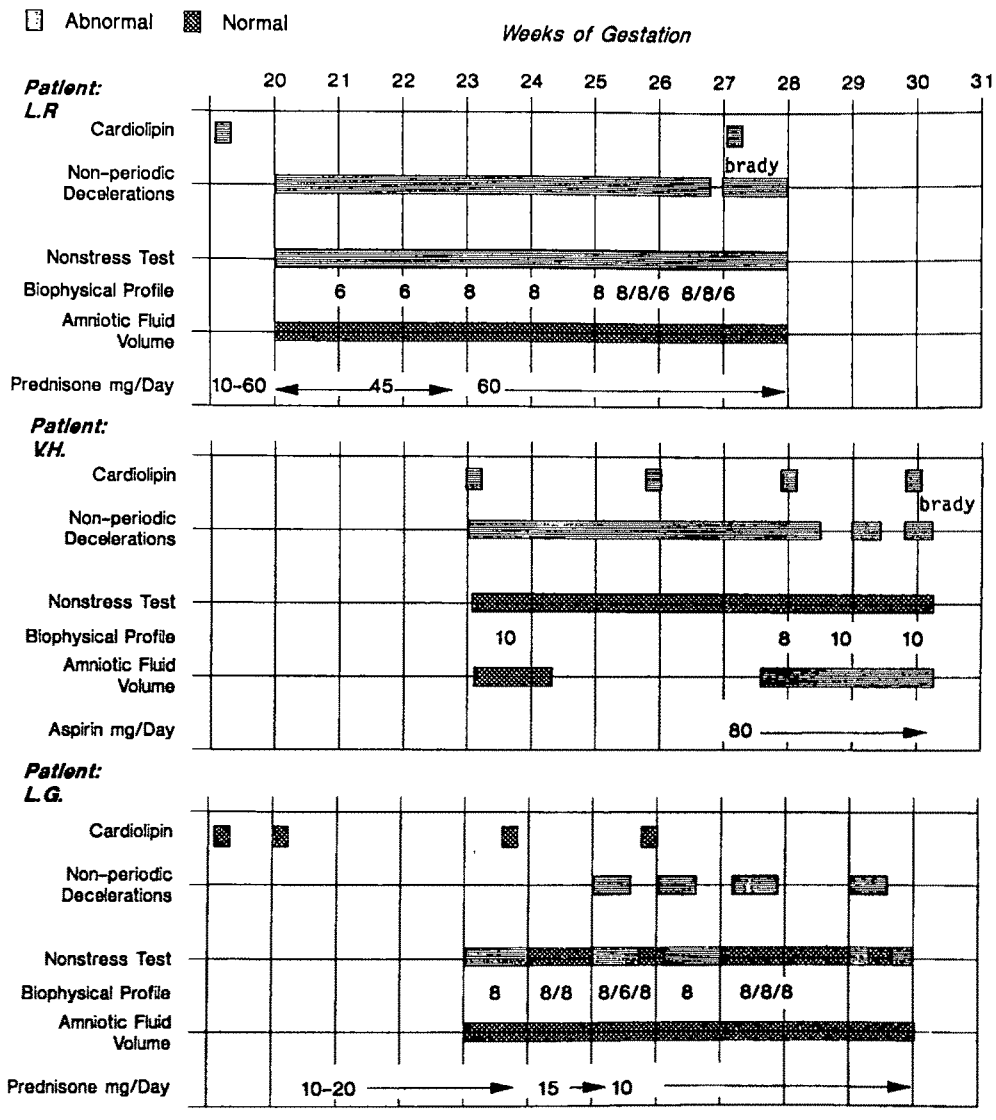


Fig. 4. Complicated lupus cases—live births.

amniotic fluid volume. Fetal bradycardia occurred in two cases and delivery took place 48 and 72 hours later (Cases Nos. 5 and 6). The mean stay in the neonatal intensive care unit for these three neonates was 85 days. Ventilator support because of respiratory distress syndrome was not required in any case.

One of the small for gestational age newborn infants (Case No. 4) developed respiratory syncytial virus pneumonia and was placed on the respirator for 3 days at 22 days of life. She was discharged after 88 days. At 26 months, this child had developed normal neurological and developmental patterns. Both height and weight were below the fifth percentile.

The second small for gestational age neonate (Case No. 5) had an uneventful course and was discharged from the hospital after 103 days. Follow-up at 4½ months revealed hypertonia, prominent primary reflexes, and increased extension. Both height and weight, corrected to 2 months, were below the third percentile.

The third neonate (Case No. 6) had necrotizing enterocolitis at 21 days of life. The infant recovered from this episode and was discharged after 64 days' stay in the intensive care unit. At 10 months of age this infant has reached normal milestones.

Group 2. No nonperiodic decelerations or bradycardias were noted in the three uncomplicated pregnancies with systemic lupus erythematosus. All pregnancies went to term and all Apgar scores were >6. However, all three patients underwent cesarean section, one because of fetal distress, one because of maternal thrombocytopenia with circulating antiplatelet antibodies,

Table IV. Laboratory data

Case No.	*Antibody to cardiolipin*	*Estimated gestational age (wk)*	*Partial thromboplastin time*	*Lupus anticoagulant*	*False positive VDRL test results*	*Thrombocytopenia*
Group 1						
1	Normal	13-20	Normal	No	No	Yes
2	Abnormal	6-25	Abnormal	Yes	Yes	No
3	Abnormal	6	Normal	No	No	No
4	Abnormal	6-28	Normal	No	No	Yes
5	Abnormal	13-30	Normal	No	No	No
6	Normal	6-26	Normal	No	No	Yes
Group 2						
1	Abnormal	4-40	Normal	No	No	No
2	Abnormal	11-36	Normal	No	Yes	Yes
3	Not available		Normal	No	No	No

and one because of cephalopelvic disproportion. The second infant was small for gestational age.

Group 3. All six control patients had term, uncomplicated deliveries. All Apgar scores were >6.

Comment

High levels of antibody to cardiolipin are a sensitive and specific predictor of fetal death or fetal distress. However, levels of antibody to cardiolipin cannot be used to determine the timing of elective premature delivery. The presence of nonperiodic decelerations in the second trimester may be an early indicator of fetal compromise in a select group of patients with a high risk of poor fetal outcome. These decelerations occurred long before traditional abnormal ultrasound findings such as oligohydramnios. These decelerations were noted only in the pregnancies with complicated systemic lupus erythematosus in which five of the six infants were small for gestational age. When intervention in the form of delivery was not accomplished, fetal death occurred. Fetal bradycardia was the terminal pattern in two of three cases. In the three cases in which intervention by elective delivery was accomplished, neonatal survival was 100%. Fetal bradycardia prompted delivery within 72 hours in two of three cases. In this select group of patients, the high incidence of nonreactive nonstress tests at an early gestational age did not add to the predictive reliability of the test.

In summary, nonperiodic decelerations of the fetal heart rate occurring in the second trimester in pregnancies complicated by systemic lupus erythematosus/circulating anticoagulant may be an indicator of intrauterine compromise. Bradycardia in these cases strongly suggests impending fetal death. Active intervention in terms of elective delivery may prevent fetal death. Other indicators of fetal compromise such as antibody to cardiolipin, oligohydramnios, and reactivity do not provide the obstetrician with the day-to-day information required to time an elective delivery.

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Vaginal birth after cesarean

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Allowing a woman with a previous cesarean birth a trial of labor rather than performing an elective repeat cesarean section continues to be a controversial area in obstetrics today. In an effort to evaluate the risks associated with a trial of labor, a prospective investigation was undertaken from July 1, 1982, through June 30, 1984. During the first year of the study, patients with a known vertical scar or more than one prior cesarean birth were excluded from an attempted trial of labor. Beginning July 1, 1983, patients with two prior cesarean births were no longer excluded and were studied prospectively. During this 2-year period, 32,854 patients were delivered of their infants at the Los Angeles County/University of Southern California Medical Center. Of these patients, 2708 (8.2%) had undergone a prior cesarean birth, and 1796 women (66%) underwent a trial of labor. A total of 1465 (81%) of them achieved a vaginal delivery. Successful vaginal delivery by the number of prior cesarean sections was as follows: one, 82%; two, 72%; three, 90%. When contrasted with the group without a trial of labor, the group with a trial of labor had significantly less maternal morbidity. In a comparison of the groups with and without a trial of labor, the incidence of uterine dehiscence (1.9% versus 1.9%) and rupture (0.3% versus 0.5%) was similar. With the application of attempted vaginal delivery in our patients with a previous cesarean birth, we were able to reduce our cesarean delivery rate for this population alone by 54%. In summary, the benefits associated with a trial of labor in the patient with a prior cesarean birth far outweigh the risks. The policy of "once a cesarean section, always a cesarean section" should be abandoned. (Am J Obstet Gynecol 1987;157:1510-5.)

Key words: Trial of labor, cesarean delivery, uterine dehiscence

The cesarean delivery rate has continued to climb during the past decade.[1, 2] Reasons for this rise have been related to the current management of the breech presentation, the identification of fetal distress, and the management of arrest disorders during labor. Attempts to reduce the cesarean delivery rate have included external version of the breech[3] and transverse lie[4] presentations and fetal scalp[5] and acoustic[6] stimulation as an alternative to fetal scalp sampling in cases of suspected intrapartum fetal distress. While these clinical techniques are effective, their impact on reducing the cesarean section rate is small. One policy that contributed approximately 30% to this rise in cesarean birth rate is the performance of elective repeat cesarean section on the basis of a lower uterine segment scar. The National Institutes of Health Concensus Development Task Force on Cesarean Birth in 1981 suggested an evaluation of this policy to determine whether vaginal birth after cesarean section was a reasonable alternative to our traditional approach.[2]

A previous retrospective investigation from our institution demonstrated that patients with one prior cesarean birth can safely be permitted a trial of labor.[7] On the basis of this review, a prospective investigation was undertaken to determine the clinical efficacy and

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Table I. Study criteria for patients with previous cesarean section during current investigation period, 1982 to 1984

Inclusion	
Patient acceptance	
No. of prior cesarean sections	
1982-1983	1
1983-1984	2
Unknown type of scar	
Exclusion	
Known classical scar	
Multiple gestation	
Malpresentation	

safety of permitting patients with one prior cesarean section a trial of labor.[8] The purpose of this report is to augment previous observations and detail our experience in patients with two prior cesarean sections who underwent a trial of labor.

Material and methods

The study population consisted of those patients who were delivered of their infants at the Los Angeles County/University of Southern California Medical Center, which is the teaching hospital for the University of Southern California. The inclusion criteria are depicted in Table I. Before an attempted vaginal delivery, patients were informed of the risks, benefits, potential complications, and alternatives to a trial of labor after a prior cesarean section. After acceptance by the patient, each one underwent a trial of labor. If a patient refused attempted vaginal delivery, a repeat cesarean delivery was planned. Entry criteria were different for each year of the study. In the first year, the emphasis was on the safety of permitting patients with one prior cesarean birth a trial of labor, whereas the second year of the study included a trial of labor in patients with two prior cesarean births. Exclusion criteria for both study years included patients with a known classical cesarean section scar, multiple gestation, or malpresentation. On the basis of our prior experience described by Beall et al.,[9] patients with an unknown type of scar were allowed a trial of labor. Of note, patients with a low vertical uterine scar were not excluded from a trial of labor.

During labor, the fetal heart rate and uterine activity were continuously monitored with a direct fetal scalp electrode and an intrauterine pressure catheter in most instances. In each patient, blood was available, an intravenous line was established, oxytocin was administered according to The American College of Obstetricians and Gynecologists' guidelines,[10] and epidural anesthesia was induced when indicated. Routine nursing care was provided to all patients.

After delivery, a uterine exploration was done in an attempt to identify any dehiscence in those patients who achieved a vaginal delivery. For the purpose of this investigation, uterine dehiscences were subcategorized according to the need for operative intervention. Scar separations that required no intervention were termed dehiscences; those that did require intervention were termed uterine ruptures.

Data were collected from the patient's chart and logbooks while the patient remained in the hospital. Statistical evaluation was by χ^2 analysis.

Table II. Incidence of trial of labor in patients with prior cesarean section for the two study periods, 1982 to 1983 and 1983 to 1984

	1982-1983[7]		*1983-1984*	
	n	%	*n*	%
Total patients	1244		1464	
Trial of labor				
Attempted	751	60	1045	71
Successful	614	82	851	81

Table III. Outcome of trial of labor related to indication for primary cesarean section

Indication for previous cesarean section	*No.*	*Vaginal delivery*	
		n	%
Arrest of labor	812	622	77*
Breech presentation	332	294	89*
Fetal distress	168	140	83
Other	484	409	85

*Vaginal birth rate after prior cesarean section for arrest of labor as compared with breech presentation: $p < 0.05$.

Results

During the 2-year period, 32,854 patients were delivered of their infants at our institution. Of these patients, 2708 (8.2%) had undergone a prior cesarean section. Forty-one patients with an unknown intent as to a trial of labor and 24 patients with multiple gestations were excluded from the study. The remaining 2643 patients form the basis for this report.

The incidence of trial of labor for the two study years is illustrated in Table II. During this time, 170 women (8.5%) who satisfactorily met entry criteria did not desire a trial of labor and underwent a repeat cesarean section. For the study periods, the incidence of a trial of labor was 60% and 71%, respectively. A total of 1796 patients (66%) underwent a trial of labor. This represented a 15% increase during the second year that was accounted for by the threefold increase in the number of patients with two prior incisions who elected a trial

Table IV. Morbidity measures and hospital stay: Comparisons in study groups

	Trial of labor (N = 1796)				*No trial of labor (N = 847)*					
	Success (n = 1465)		*Failure (repeat cesarean section) (n = 331)*		*Vaginal delivery (n = 69)*		*Elective cesarean section (n = 314)*		*Other indication for cesarean section (n = 464)*	
Morbidity	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Dehiscence	22	1.5	17	5.1	3	4.3	7	2.2	10	2.2
Febrile morbidity	53	3.6	106	32	4	5.8	56	18	103	22
Hospital days	2.2		4.2		2.3		4.2		4.2	

Table V. Maternal morbidity related to trial of labor contrasted with no plan for trial of labor

	Trial of labor (N = 1796)		*No trial of labor (N = 847)*	
Maternal morbidity	*n*	%	*n*	%
Dehiscence	34	1.9	16	1.9
Uterine rupture	5	0.3	4	0.5
Febrile morbidity	159	8.9*	163	19.2
Hysterectomy	5	0.3*	14	1.7

*$p < 0.05$.

Table VI. Incidence of oxytocin usage for the two study periods

	1982-1983[16]		*1983-1984*	
	n	%	*n*	%
No. with trial of labor	751		1045	
Oxytocin usage	289	38	504	48
Vaginal delivery	200	69	357	71

of labor. At the same time, the precentage of patients achieving a vaginal delivery was similar for both years (81%). Indications for repeat cesarean delivery after a trial of labor were: arrest disorders, 237 (72%); fetal distress, 48 (15%); and other indications, 46 (13%). During the course of labor, epidural anesthesia was induced in 244 (14%) of the patients.

Outcomes of trial of labor as they relate to previous indications are depicted in Table III. As previously demonstrated,[11] those patients with the greatest likelihood of successful vaginal delivery were those patients whose prior cesarean section indication was breech presentation. Those patients least likely to achieve a vaginal delivery were those whose previous cesarean section was done for cephalopelvic disproportion–failure to progress. While the latter group had the least success, more than 75% of these patients were delivered vaginally. Fetal distress as an indication for primary cesarean section was not found to be a recurrent cause for a repeat cesarean birth during a subsequent trial of labor.

A comparison between those patients undergoing a trial of labor with those not undergoing such a trial demonstrated some marked similarities and differences between the two groups. The risk of uterine dehiscence, while insignificantly increased in the patients with a failed trial of labor (Table IV), was similar for both the overall trial of labor and no trial of labor groups (Table V). According to standard febrile morbidity criteria, the failed trial of labor group had the greatest incidence of febrile morbidity (Table IV); and this rate of febrile morbidity was comparable to that of our primary cesarean section population. However, the overall trial of labor group had significantly less febrile morbidity (Table V) and a shorter hospital stay (Table IV).

Oxytocin administration during an attempted vaginal delivery remains controversial. Our results are illustrated in Table VI. During the second year of the study, oxytocin administration increased 26% over that of the previous year, and oxytocin was given to 793 patients (44%) undergoing a trial of labor. The indications for oxytocin use were induction of labor in 59 patients (7%) and augmentation of labor in 734 patients (93%). Irrespective of the indication for oxytocin, successful vaginal delivery was achieved in 70% of the patients who received oxytocin compared with 91% (908/1003) of patients who did not receive oxytocin. When patients who received oxytocin were contrasted with those who did not, the incidence of repeat cesarean section for fetal distress (3% for each) and the rate of uterine dehiscence (3% and 2%) were comparable. The combination of oxytocin and epidural anesthesia was used in 178 (22%) patients. This combination was associated with a cesarean delivery rate of 31%, which is comparable to that of patients who received oxytocin alone. Moreover, the incidence of uterine dehiscence was also similar.

During the second year of the investigation, the major emphasis was on permitting patients with two prior

Table VII. Outcome of trial of labor related to number of prior cesarean deliveries

No. of prior cesarean sections	1982-1983[7]					1983-1984				
		Trial of labor		Vaginal delivery			Trial of labor		Vaginal delivery	
	No.	n	%	n	%	No.	n	%	n	%
1	923	726	76	595	82	1111	911	82	753	83
2	226	23	10	16	70	260	126	49	91	72
≥3	60	2	3	2	100	62	8	13	7	88

cesarean sections a trial of labor. The number of patients with two and three cesarean births undergoing a trial of labor increased threefold (Table VII). The likelihood of successful vaginal delivery according to the number of prior cesarean sections was as follows: one, 1348/1637 (82%); two, 107/149 (72%); and three, 9/10 (90%). The rates of oxytocin administration (50%) and the use of epidural anesthesia (15%) were also similar for patients with one and two or more cesarean sections.

Maternal morbidity relating to a trial of labor is depicted in Table V. Uterine dehiscences and rupture were similar for both study groups. In cases of uterine dehiscence, the patients usually had no clinical signs or symptoms. However, if the umbilical cord prolapsed through a dehiscent lower uterine segment or a uterine rupture occurred, these events were frequently heralded by signs of fetal distress such as severe variable fetal heart rate decelerations or a prolonged fetal heart rate deceleration. Of note, maternal pain or changes in uterine tone usually were not observed. During a trial of labor, one significant uterine rupture occurred. This patient, a 32-year-old woman, gravida 4, para 1-0-2-1, with a prior cesarean section for cephalopelvic disproportion, presented in spontaneous labor with an estimated fetal weight of 3800 gm at 40 weeks' gestation. During the course of labor, she received increasing doses of oxytocin for a protracted active phase. With the cervix at 9 cm, a sudden prolonged fetal heart rate deceleration was observed and an immediate cesarean delivery was performed. A 4600 gm infant was delivered with Apgar scores of 0, 0, and 3 at 1, 5, and 10 minutes, respectively. Findings included a posterior uterine wall rupture and an intact low transverse scar. The uterine wall defect was repaired. At the time of and before the fetal heart rate deceleration, the patient was not under epidural anesthesia, and an intrauterine pressure catheter was in place. Except for the deceleration, no classic signs or symptoms of uterine rupture were present. While the maternal postpartum course was benign, the neonatal course was complicated, ultimately leading to the death of the infant. This represents the only fetal death directly attributable to a trial of labor. It is important to note that this death could have occurred in any labor and was a labor-related death rather than having to do with her risk status because of a uterine scar.

Table VIII. Indications for hysterectomy in patients with prior cesarean birth (N = 19)

Indication	Trial of labor	No trial planned
Atony	5	6
Placenta accreta	0	5
Uterine laceration	0	2
Elective	0	1

Overall, 19 hysterectomies (0.7%) were performed in the entire study group (Table VIII). Of these, five (0.3%) were performed because of uterine atony in patients who underwent a trial of labor. Of the 14 hysterectomies performed in the group without a trial of labor, six were done because of uterine atony and hemorrhage. Hysterectomy was significantly more common in the group without a trial of labor. No patient who was delivered vaginally underwent a hysterectomy.

In the study population, there was a single maternal death caused by an autopsy-confirmed postpartum pulmonary embolus ina patient who was allowed a trial of labor but who underwent a cesarean section because of fetal distress.

The perinatal outcome for the entire study population consisted of 17 fetal deaths and 23 neonatal deaths, for a perinatal mortality rate of 15/1000 births. This perinatal mortality rate is slightly less than the overall institutional rate of 18/1000. Of the 17 fetal deaths, seven were thought to be in term fetuses (≥38 weeks) and 10 in preterm fetuses (<38 weeks). The causes of the fetal deaths at term were unknown in six instances and one fetus was noted to be anomalous. In those deaths judged to be in preterm fetuses, the causes of death were: unknown, three; anomalous, two; prolapsed cord, one; abruptio placentae, one; uterine rupture, two; intrapartum death, one. With the exception of one patient with a 4350 gm infant who was delivered by repeat cesarean section because of arrest of dilatation, those patients with a fetal death were delivered

vaginally. The two fetal deaths associated with a major uterine rupture have been previously described in detail.[7]

Of the 23 neonatal deaths, eight occurred in infants who weighed <750 gm. Of the remaining 15 neonatal deaths, 11 were in infants with multiple anomalies incompatible with life. In the remaining four patients with infant birth weights in excess of 750 gm the causes of death were: respiratory distress syndrome, one; severe erythroblastosis fetalis, one; perinatal asphyxia, two. In the two cases of perinatal asphyxia, one has been previously described in association with the posterior uterine wall rupture. The second case was in a patient with an abnormal fetal heart rate pattern and an abnormal fetal scalp pH. She underwent immediate cesarean delivery and was delivered of a 3300 gm infant with Apgar scores of 1 and 1 at 1 and 5 minutes, respectively. The baby had a complicated neonatal course and died on the eleventh day of life.

In summary, 11 of the 40 perinatal deaths occurred in infants with birth weights <750 gm. Of the remaining 29 deaths, 14 occurred in anomalous infants. In the 16 other deaths, the pregnancies were judged to be term in 10 and preterm (<38 weeks) in six. Four cases of fetal death were excluded: preterm uterine rupture, two; abruptio placentae, one; and cord prolapse, one. The corrected perinatal mortality rate for the study population is 4.5/1000.

Comment

An increasing familiarity with trial of labor in the patient with a scarred uterus has led us to increase our incidence of trial of labor, in 1983 and 1984, to 71% of patients with a prior cesarean section. Overall, 65% of our patients agreed to undergo a trial of labor. This is in marked contrast to previous reports in which the incidence of acceptance was in the range of 50% to 60%.[12-14] The two factors primarily responsible for this increase have been our policy of permitting patients with an unknown type of scar and our recent policy of allowing patients with two or more prior cesarean sections a trial of labor.

Previous indication for cesarean birth bears only little relationship to the subsequent successful vaginal delivery. Our data continue to show that those patients with the greatest likelihood of vaginal delivery are those with a prior cesarean section done because of breech presentation. Additionally, more than 75% of those patients whose prior cesarean section was done because of cephalopelvic disproportion still achieved a vaginal delivery. Irrespective of the indication for prior cesarean birth, the factors associated with an enhanced probability of vaginal delivery were a prior vaginal birth[7] and an infant with a birth weight <4000 gm.[15]

On the basis of our experience in 793 patients who underwent either induction or augmentation of labor, we believe that the use of oxytocin is a reasonable consideration in the patient with a previous cesarean birth. As is the case in the patient with an unscarred uterus, the administration of oxytocic agents should be precise, by the use of infusion pumps and coupled with careful electronic monitoring of both mother and fetus. The risk of uterine dehiscence or rupture was not significantly increased in patients receiving oxytocin. However, as previously demonstrated by Horenstein and Phelan,[16] the use of oxytocin during a trial of labor is associated with a significantly greater likelihood that a cesarean birth will need to be performed. Additionally, the use of epidural anesthesia was not associated with a greater likelihood of cesarean delivery or maternal or fetal morbidity.

The overall incidence of uterine dehiscence was 2.2%, and the incidence was similar in patients undertaking a trial of labor and in those in whom no trial of labor was planned. The apparently higher rate of dehiscence diagnosed at repeat cesarean section is more likely due to the ability to directly observe the scar. After vaginal birth, transcervical examination of the scar is more difficult and less likely to detect a subtle dehiscence. Most important, the incidence of uterine dehiscence was unrelated to the use of oxytocin, epidural anesthesia, the previous indication for cesarean section, and the number of prior cesarean births. It is important to note that uterine ruptures in the current series were associated with prior fundal or classical incisions and not lower-segment incisions; as such, we would continue to recommend that patients with a known fundal or classical uterine incision not be permitted a trial of labor.

The management of patients with a uterine defect identified at vaginal delivery is somewhat controversial. However, we adhere to our prior recommendation that nonbleeding defects in the lower uterine segment and in a hemodynamically stable patient do not need to be repaired. If there is significant bleeding, if the patient's condition is unstable, or if the defect is in the upper segment, exploratory laparotomy would be warranted and repair as indicated. The question of route of delivery in a subsequent pregnancy after the observation of a dehiscence or low-segment defect after vaginal delivery remains unanswered. While one patient in the current series with a uterine "window" did undergo an uncomplicated vaginal delivery, we would recommend elective repeat cesarean delivery in these patients.

Previous investigators have suggested that patients with more than one previous uterine incision can be safely permitted a trial of labor.[17] As demonstrated in Table VII, 72% of our patients with two prior cesarean sections who underwent a trial of labor were delivered vaginally. During labor, 48% of these patients required

oxytocin for augmentation of labor. It appears that the patient who has a primary cesarean section because of cephalopelvic disproportion and subsequently has failure of a trial of labor for the same reason has the greatest likelihood of requiring a repeat cesarean section for the same indication.

Whether the patient with an unknown type of uterine scar should be permitted a trial of labor is highly controversial. A prior report by Beall et al.[9] and the current investigation would appear to support permitting these patients a trial of labor. While more detailed studies are needed to address this question, a trial of labor in the patient with an unknown type of scar would not appear unreasonable. However, before a trial of labor is undertaken in these patients, consultation with a perinatologist and informed consent from the patient should be obtained.

Traditionally, patients with a previous cesarean birth undergo an elective repeat cesarean delivery around 38 weeks' gestation. An evaluation of our perinatal mortality statistics showed that six unexplained stillbirths occurred after 38 weeks' gestation. A similar finding was also observed by Gibbs.[18] Theoretically, these fetal deaths could have been prevented by the traditional approach of elective repeat cesarean section. As a result, patients who elect to attempt vaginal delivery after cesarean section are exposed to a greater risk of fetal death, although no greater than that of a normal population at a similar gestational age, than the patient who elects a repeat cesarean birth. While more data are needed, one consideration, in an attempt to minimize the risks of fetal death while the patient awaits the onset of labor, is antepartum fetal surveillance beginning at or around 38 weeks.

A prior retrospective analysis by Boucher et al.[19] demonstrated that patients with a prior cesarean section who underwent a trial of labor had significantly less maternal morbidity. The current investigation corroborates this observation. Overall, there was a significant decrease in both febrile morbidity and emergency hysterectomy. However, a failed trial of labor was associated with an increased risk of febrile morbidity (Table IV). While one maternal death did occur in the current investigation, this was secondary to a massive pulmonary embolus and unrelated to the trial of labor.

In summary, by permitting a trial of labor in our population with a previous cesarean section, we were able to reduce our cesarean delivery rate by 54% without a significant increase in fetal or maternal morbidity. Overall, our cesarean section rate was reduced 4.5%. Oxytocin may be safely used for the usual obstetric indications as long as one adheres to The American College of Obstetricians and Gynecologists' guidelines for oxytocin usage.[10] Third, in the patient who has had a prior cesarean birth and has reached a gestational age of 38 weeks, routine fetal surveillance may be a reasonable consideration. Fourth, in patients with two prior cesarean sections, a trial of labor appears to be a reasonable alternative. Last, our data support the view that the policy of "once a cesarean section, always a cesarean section" should be abandoned.

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The reliability and clinical use of a rapid phosphatidylglycerol assay in normal and diabetic pregnancies

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Lecithin phosphorus concentration, the standard fetal lung maturity test in our institution, and phosphatidylglyercol were assayed in 69, 29, and 45 amniotic fluid samples from normal (GI), gestational (GII), and insulin-dependent diabetic (GIII) women by means of thin layer chromatography and Amniostat-FLM, respectively. Lecithin phosphorus concentration ≥0.1 mg/dl and positive or strong positive Amniostat-FLM results were considered mature. The results of both assays were concordant in 79% of the samples. The discordance rate was highest in GIII patients. In our experience, respiratory distress syndrome did not develop in neonate infants of diabetic women delivered after a mature lecithin result. With lecithin phosphorus concentration as the reference standard, the predictive value of a mature Amniostat-FLM result was 96.2%, whereas that of an immature result was 58.5%. Respiratory distress syndrome occurred in only two GIII neonates who were delivered within 72 hours of both immature lecithin and Amniostat-FLM results. These findings support the use of Amniostat-FLM as a screening test for fetal lung maturity in both normal and diabetic pregnancies. Additional tests will be necessary to evaluate further fetal lung maturity only if the results are negative. (AM J OBSTET GYNECOL 1987;157:1516-20.)

Key words: Amniostat-FLM, lecithin phosphorus concentration, fetal lung maturity, normal and diabetic pregnancies, respiratory distress syndrome

The risk of the development of respiratory distress syndrome (RDS) was reported to be higher in infants of diabetic mothers. It has been recommended that fetal lung maturity studies be performed in diabetic patients and that delivery should not be planned except after documentation of fetal lung maturity.[1] The lecithin/sphingomyelin ratio is the most widely used fetal lung maturity test.[2] At the Medical College of Georgia Hospital, the amniotic fluid concentration of lecithin, which is expressed as its phosphorus content, is measured, and a value ≥0.1 mg/dl indicates maturity in both normal and diabetic pregnancies.[1,3] Whereas several studies documented the occurrence of RDS in some infants of diabetic mothers even after attainment of "mature" lecithin/sphingomyelin ratios,[4,5] in our own experience, none of these infants delivered after the attainment of a "mature" lecithin result developed respiratory distress syndrome.[1,6]

The presence of phosphatidylglycerol in the amniotic fluid is believed to predict accurately the absence of RDS in the neonate in both normal and diabetic pregnancies.[7,8] In these studies, a thin layer chromatographic technique was used for the assay of phosphatidylglycerol. More recently, a rapid slide immunologic agglutination test has been developed for phosphatidylglycerol identification and semiquantitation and is commercially available, that is, Amniostat-FLM (Hana Biologic, Inc., Berkeley, California). The results of phosphatidylglycerol assay by thin layer chromatography and Amniostat-FLM were reported to be concordant in 90% to 95% of the cases.[9-11] The purpose of this study was to determine the degree of concordance between the results of Amniostat-FLM and our standard test (lecithin phosphorus concentration), to assess the accuracy of the former method in predicting lung maturity, and to determine its clinical use in both normal and diabetic pregnancies.

Material and methods

The study patients were managed and delivered at Medical College of Georgia Hospital from September 1983 to March 1985. Patients who had amniocentesis performed for clinical indications were recruited for this study. Twin pregnancies, as well as patients who had premature rupture of membranes or who had received glucocorticoid therapy, were excluded. Informed consent was obtained. All amniocenteses were performed with ultrasonic guidance.

The study included 122 patients divided into the following groups: 63 normal pregnant control subjects who also had a normal glucose screening test[12] (GI), 25 gestational diabetics as defined by the criteria of

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Table I. Amniotic fluid lecithin phosphorus concentration in the study subjects: Relationship with the results of Amniostat-FLM

	GI: Normal pregnant women	*GII: Gestational diabetics*	*GIII: Insulin-dependent diabetics*
Negative*	0.08 ± 0.04†‡	0.10 ± 0.07‡	0.12 ± 0.07‡
Positive	0.24 ± 0.14	0.23 ± 0.11	0.26 ± 0.19
Strong positive	0.46 ± 0.12	0.42 ± 0.34	0.47 ± 0.13

*Negative is considered "immature" whereas positive and strong positive are considered "mature."
†mg/dl, mean ± SD.
‡Significantly lower than the other two values in the same column, analysis of variance followed by Tukey's hsd procedure.

Table II. Concordance between Amniostat-FLM and lecithin phosphorus concentration in the samples studied

	GI: Normal (n = 69)		*GII: Gestational diabetics (n = 29)*		*GIII: Insulin-dependent diabetics (n = 45)*		*Total (n = 143)*	
Amniostat-FLM	*Mature**	*Immature*	*Mature*	*Immature*	*Mature*	*Immature*	*Mature*	*Immature*
Negative (n = 65)	7 (10)†	20 (29)	4 (14)	6 (21)	16 (36)	12 (27)	27 (19)	38 (27)
Positive and strong positive (n = 78)	41 (59)	1 (2)	19 (65)	0 (0)	15 (33)	2 (4)	75 (52)	3 (2)
Total (n = 143)	48 (69)	21 (31)	23 (79)	6 (21)	31 (69)	14 (31)	102 (71)	41 (29)

*Lecithin phosphorus concentration ≥0.1 mg/dl.
†Numbers within parentheses represent percentage within each group.

O'Sullivan et al.[12] and were diet controlled (GII), and 34 insulin-dependent diabetics (GIII), made up of 21 class B, seven class C, five class D, and one class FR patients. From these patients, 143 amniotic fluid samples that were neither meconium- nor blood-stained were obtained. Sixty-nine, 29, and 45 samples were analyzed from GI, GII, and GIII patients, respectively. On receipt, one aliquot of the amniotic fluid was analyzed for the absolute concentration of lecithin as measured by its phosphorus content by means of a previously described chromatographic technique.[13] Another aliquot was analyzed for phosphatidylglycerol by Amniostat-FLM. The manufacturer's method was used, and the results were reported as either negative (no agglutination), positive (weak agglutination), or strong positive (strong agglutination). The diabetic patients were managed according to our previously published protocol.[1, 6]

The gestational age at delivery was based on menstrual dates corroborated by sonographic parameters. This did not differ from the neonatal assessment by >2 weeks in any of the study patients. The gestational age at amniocentesis was back calculated from the gestational age at delivery.

The results of both Amniostat-FLM and lecithin phosphorus concentration were recorded as mature or immature. For Amniostat-FLM, the positive and strong positive results were recorded as mature whereas the negative results were recorded as immature. Lecithin phosphorus concentration in amniotic fluid ≥0.1 mg/dl was considered mature, and concentrations <0.1 mg/dl were considered immature. With the latter as the reference standard, the sensitivity, specificity, positive, and negative predictive values of Amniostat-FLM test were calculated. These were compared among the three groups by means of χ^2 analysis. The clinical parameters and the lecithin phosphorus concentration were compared by analysis of variance. When the F test in the analysis of variance was statistically significant ($p < 0.05$), Tukey's hsd procedure was used to test pairwise comparisons posthoc.

Result

The insulin-dependent diabetic patients were slightly but significantly older than the normal group (28.9 ± 6.7 versus 24.3 ± 5.0 years). There were no significant differences in gestational age at amniocentesis (37.7 ± 2.5, 37.8 ± 1.5, 37.0 ± 2.0 weeks in GI, GII, and GIII, respectively). The range was 29 to 42 weeks in GI, 34 to 41 weeks in GII, and 30 to 40 weeks in GIII patients. The gestational age at delivery was slightly but significantly less in GIII patients than in GI and GII patients (38.0 ± 1.8 versus 39.0 ± 1.6 and 39.0 ± 1.5 weeks, respectively).

In all three groups, lecithin phosphorus concentration was significantly higher when the Amniostat-FLM

Table III. The accuracy of Amniostat-FLM in relation to lecithin phosphorus concentration

	GI normal pregnant women	*GII gestational diabetics*	*GIII insulin-dependent diabetics*	*Total*
A = True immature B = False immature C = True mature D = False mature				
Sensitivity = $\frac{A}{A + D}$	95.2*	100	85.7	92.7
Specificity = $\frac{C}{C + B}$	85.4	82.6	48.4†	73.5
Predictive value of immature results = $\frac{A}{A + B}$	74.1	60	42.9†	58.5
Predictive value of mature result = $\frac{C}{C + D}$	97.6	100	88.2	96.2

*Percentage.
†Significantly lower than in GI.

result was either positive or strongly positive than when it was negative (Table I). There were 65 samples in which Amniostat-FLM results were immature. Of those, 27 (41.5%) samples had a mature lecithin phosphorus concentration. On the other hand, in 78 samples, the Amniostat-FLM results were mature, and the lecithin phosphorus concentration was immature in only three of these (3.9%) (Table II). The results of the two tests were concordant in 113 of 143 (79%) samples. Both were immature (true immature Amniostat-FLM) in 28 (26.5%) samples, and both were mature (true mature Amniostat-FLM) in 75 (52.5%) samples (Table II). The results of the two tests were discordant in 30 of 143 (21%) samples. The discordance rate was highest (40%) in GIII samples and was significantly higher than the discordance rate (11.6%) in GI samples ($p < 0.005$). A false immature Amniostat-FLM result occurred in 27 samples, which represented 90% (27/30) of the discordant results or 18.8% (27/143) of all samples. A false mature Amniostat-FLM result occurred in only three samples, which accounted for 10% (3/30) of the discordant results or 2.1% (3/143) of all samples (Table II).

Of the 27 samples with false immature Amniostat-FLM results, seven were GI, four were GII, and 16 were GIII. The incidence of this type of discordance was significantly higher in GIII samples: 16 of 31 (51.6%) compared with seven of 48 (14.6%) in GI and four of 23 (17.4%) in GII samples ($p < 0.005$) (Table II). Delivery within 72 hours of amniocentesis occurred in three of seven GI patients, one of four GII patients, and four of 16 GIII patients; none of the neonates developed RDS. Of the three false mature Amniostat-FLM results, two were in GIII patients and one was in a GI patient; none of these neonates developed RDS. However, it should be noted that only one of these neonates (GIII) was delivered within 72 hours.

Only two infants, both GIII, developed RDS, giving an incidence of 1.6% (2/122) for the whole study and 5.9% (2/34) for GIII. Both infants were delivered within 72 hours of an immature lecithin phosphorus concentration and a negative Amniostat-FLM result.

Amniostat-FLM results were available in 91 infants delivered within 72 hours of amniocentesis. Mature results occurred in 78 infants and none developed RDS. Only two of the other 13 infants who were delivered within 72 hours of a negative Amniostat-FLM result developed RDS. None of the 101 infants who were delivered after a mature lecithin result and only two of six neonates delivered within 72 hours of an immature lecithin concentration developed RDS.

The calculated sensitivity, specificity, and predictive value of mature and immature Amniostat-FLM test results that used lecithin phosphorus concentration as the reference standard are given in Table III. The specificity and predictive value of immature results were significantly lower in GIII patients than in GI patients.

Comment

Fetal lung maturity testing has become a very important tool in the management of high-risk pregnancies, especially diabetic pregnancies. Measurement of lecithin/sphingomyelin ratio has been used extensively.[2] At the same time, measurement of the absolute lecithin concentration, expressed as its phosphorus content, was developed at our institution.[13] The incidence of false mature lecithin/sphingomyelin ratio results continued to be a problem in diabetic pregnancies, varying from 3%[4] to 27%.[5] However, using lecithin phosphorus concentration, we did not experience false mature results.[1, 6] Nevertheless, both tests share the following same disadvantages: (1) The methodology is sophisticated; (2) they need specially trained personnel; (3) they

are time-consuming; (4) they are affected by the presence of blood or meconium; (5) vaginally collected amniotic fluid samples cannot be reliably used; and (6) they are not available on a 24-hour basis in most clinical laboratories.

Certainly, the addition of phosphatidylglycerol analysis has reduced the incidence of false predictions of the absence of RDS. Moreover, phosphatidylglycerol results are reliable when amniotic fluid samples are contaminated with blood or meconium or when they are collected from vaginal pools. However, the assay of phosphatidylglycerol by means of two-dimensional thin layer chromatography[7-11, 14] is labor intensive, requires considerable technical expertise, and takes a long time (3 to 4 hours) to perform.

More recently, Amniostat-FLM was developed to determine the presence of phosphatidylglycerol in the amniotic fluid by a rapid slide immunologic agglutination method that only takes 20 to 30 minutes to perform. Garite et al.[9] found that the results of phosphatidylglycerol assayed by thin layer chromatography and the agglutination method were concordant in 91% of the samples. However, the quantities of reagents used and test conditions were slightly different from those in the Amniostat-FLM kit that we used. In two subsequent studies[10, 11] in which this kit was used, the concordance rates were 90.1% and 85%, respectively. With the lecithin/sphingomyelin ratio as the reference standard, when it was mature, Amniostat-FLM was mature in 78.4% of samples, whereas when it was <2.0, 44 of 47 (93.6%) samples were also immature by Amniostat-FLM.[10] Halverson and Gross[11] reported that of the 154 samples in which the lecithin/sphingomyelin ratio was mature, 31 samples were negative by Amniostat-FLM, whereas of the 26 samples in which the lecithin/sphingomyelin ratio was <2.0, three were positive by the Amniostat-FLM method. These results are comparable with ours. However, in two of these studies,[9, 10] there was no indication of how many diabetic patients (if any) were included. In the third study[11] there were only 11 class A and 29 insulin-dependent diabetic patients, and no attempt was made to separate the results of diabetic patients from those of the other study patients.

No previous studies compared the results of lecithin phosphorus concentration to that of phosphatidylglycerol, whether it was assayed by thin layer chromatography or by Amniostat-FLM. Using Amniostat-FLM, we found the results to be concordant in 79% of the samples. The sensitivity and specificity of Amniostat-FLM in relation to lecithin phosphorus concentration compared favorably with previously reported results that used lecithin/sphingomyelin ratio as the reference standard. The discordance rate was significantly higher in insulin-dependent diabetic patients than in the normal group, which concurs with previous reports that compared phosphatidylglycerol assayed by thin layer chromatography with lecithin/sphingomyelin ratio. Although the concordance rate in diabetic patients was about the same in these studies (56%)[8, 14] as in our (60%), the discordance rate in normal pregnant women in the present study was lower (12%) than in the other two studies—30%[14] and 40%.[8]

The incidence of RDS in our insulin-dependent diabetic patients was low (5.9%). This is the result of our management protocol, which emphasizes tight diabetic control and documentation of fetal lung maturity before planned delivery.[1, 6, 15] It also confirms the accuracy of the fetal lung maturity test we have used, that is, lecithin phosphorus concentration. We have shown that when lecithin phosphorus concentration or Amniostat-FLM was mature, RDS did not occur. On the other hand, when lecithin phosphorus concentration was <0.1 mg/dl, only two of six neonates developed RDS, and when Amniostat-FLM was immature, only two of 13 neonates developed RDS. In the previously cited studies, only eight of 21[9] and five of 19[11] neonates who were delivered within 72 hours of a negative Amniostat-FLM result developed RDS. It is clear that there were no cases of RDS in neonates delivered after a positive Amniostat-FLM result either in our study or the previous studies (total of 231 cases). It is equally clear that a negative Amniostat-FLM does not accurately predict the occurrence of RDS, a false-negative rate of 84.6% in our study, and 62%[9] and 73.7%[11] in the other studies.

Our findings indicate that Amniostat-FLM is a safe screening test for fetal lung maturity in both normal and diabetic pregnancies. For a screening test, one would prefer that which errs on the side of false immaturity so that the obstetrician does not deliver a patient based on a falsely mature value.

An additional point merits comment. In this study, four neonates were delivered within 72 hours of a mature lecithin phosphorus and negative Amniostat-FLM result, and none of these infants developed RDS. This emphasizes the point that sometimes undue delay in the delivery of a diabetic patient with a mature lecithin phosphorus concentration because of the absence of phosphatidylglycerol (as determined by a negative Amniostat-FLM) may not be warranted and may be unwise if there is suspicion or evidence of fetal compromise. This is particularly significant because our results show a higher incidence of this type of discordance in insulin-dependent diabetic patients (Table III).

In summary, we conclude that Amniostat-FLM is an accurate, simple, and rapid test of fetal lung maturity. It is safe to use as a screening test in both normal and diabetic pregnancies. It needs to be backed up by another standard test, for example, lecithin/sphingomyelin ratio or lecithin phosphorus concentration, only if the result is negative (immature), since these tests increase the predictive accuracy of the immature results. The use of a similar stepwise approach with an initial

simple screening test has also been recommended by others.[16] However, these authors used the shake test as their primary screening test, to be followed, if negative, by fluorescence polarization and then by the lecithin/sphingomyelin ratio. There was also no indication if diabetic patients were included in their study, and if so, whether the scheme was as effective in them.[16]

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Correction

In the article by Blackwell and Garrison, entitled "Inhibition of prolactin secretion by antiserum to the α- and β-subunits of gonadotropin" (Am J Obstet Gynecol 1987;156:863-8), on p. 864, Table I did not contain the braces indicating a lack of significant difference. The corrected Table I appears below.

Table I. Effect of antisera to α-subunits of FSH and LH on basal and GnRH-stimulated prolactin secretion (N = 3)

Treatment	*Prolactin (ng/ml, mean ± SEM)*
Control	65 ± 7
Antiserum to α-subunit of LH	73 ± 15
Antiserum to α-subunit of FSH	39 ± 8
GnRH, 10^{-5} mol/L	160 ± 15
GnRH + antiserum to α-subunit of LH	150 ± 14
GnRH ± antiserum to α-subunit of FSH	146 ± 8

Values within each brace are not significantly different from each other.

Fetal umbilical artery flow velocimetry in postdate pregnancies

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This study prospectively examined the use of umbilical artery flow velocimetry for monitoring fetal health in postdate pregnancies. Forty-six patients with well-established dates were evaluated with semiweekly biophysical profiles and umbilical artery flow velocimetry (characterized by the ratio of the peak systolic to end-diastolic velocity). Their labor records were reviewed, and neonates were examined for signs of postmaturity. Twenty neonates had an abnormal test result or outcome (identified as an abnormal nonstress test, oligohydramnios, meconium, intrapartum fetal distress, or a 5-minute Apgar score <7). Nine neonates had a physical examination consistent with the postmaturity syndrome. Twenty-one neonates were entirely normal. Comparisons of the mean systolic/diastolic ratios for neonates with and without the complications associated with postdatism showed no significant differences. In addition, all systolic/diastolic ratios were within the normal range. Therefore, umbilical artery flow velocimetry is unlikely to be useful for the routine antenatal assessment of the postdate fetus. (AM J OBSTET GYNECOL 1987;157:1521-3.)

Key words: Postdates, umbilical artery velocimetry, fetal well-being

In 1951 Clifford et al.[1] reported that postmaturity of 300 days or more accounted for 30% of perinatal deaths, most of which occurred in utero. Since then, a significant decrease in perinatal deaths has been realized partly because of improvements in antepartum fetal testing. However, the increased incidence of fetal distress, the postmaturity syndrome, and meconium aspiration still contribute to neonatal morbidity. The mechanism of fetal and neonatal injury associated with the postdate pregnancy has been ascribed to progressive placental insufficiency. Histologic examination of the postdate placenta has shown an increase in the thickness of the trophoblastic basement membrane, reduction in the intervillous space caused by thrombosis with fibrin deposition, and degeneration of villi.[2]

Recently, a noninvasive evaluation of placental function has become possible by recording the umbilical artery velocity waveform by means of Doppler ultrasonography. This technique provides an indirect measure of the vascular resistance downstream from the point of measurement. An increase in the resistance to flow in the fetal villous circulation results in a decrease in the end-diastolic velocity of the umbilical artery. Various investigators have used this technique to study placental insufficiency that results in intrauterine growth retardation.[3-5] Their findings have proved useful in identifying these growth-retarded fetuses. Other investigators have advocated the application of umbilical artery flow velocimetry as a screening test to evaluate pregnancies at risk for fetal compromise.[6] The purpose of this study was to examine the ability of umbilical artery flow velocimetry to identify those fetuses at risk for adverse perinatal outcome in postdate pregnancies.

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Material and methods

One hundred patients with singleton gestations were evaluated prospectively at the Maternal Fetal Assessment Center of the Albert Einstein College of Medicine beginning at 41 weeks of gestation. These patients had reliable dates, as established by last menstrual period consistent with an early clinical examination or by last menstrual period consistent with a first-trimester sonogram. Antenatal assessment of these patients was performed twice weekly until delivery and included the following: (1) umbilical artery flow velocimetry: The waveforms, obtained with the use of a continuous-wave Doppler machine (Angioscan 3, Ultrasonics, Yonkers, N.Y.), were characterized by the ratio of the peak systolic to the end-diastolic velocity. Three or more waveforms obtained during nonbreathing periods were averaged. Normal range was defined as peak systolic to end-diastolic ratio <3[7]; and (2) a biophysical profile as described by Manning et al.[8]

Forty-six of these 100 pregnancies lasted more than 294 days and had a final assessment within 4 days of delivery. Thus these patients made up the study group. Their labor records were reviewed, and the neonates were examined by one of the authors (D. G.) in con-

Table I. Peak systolic to end-diastolic ratios for normal neonates, those with signs of postmaturity, and those with abnormal test results or outcome

Group	N	Peak systolic to end-diastolic ratio		p
		Mean ± SD	Range	
Neonates with no abnormal test result, outcome, or signs of postmaturity	21	2.07 ± 0.20	1.70-2.49	
Neonates with a physical examination showing signs of postmaturity	9	2.07 ± 0.26	1.73-2.60	NS
Neonates with				
Abnormal nonstress test	7	1.95 ± 0.13	1.73-2.10	
Oligohydramnios	3	2.17 ± 0.39	1.88-2.73	
Moderate or thick meconium	13	2.03 ± 0.33	1.64-2.83	NS
Intrapartum fetal distress	4	1.97 ± 0.16	1.73-2.16	
Apgar <7 at 5 min	1	1.73 ± 0.00	—	
Neonates with one or more abnormal test result or outcome	20	2.05 ± 0.32	1.64-2.83	NS

junction with the pediatric staff for signs of postmaturity.

An abnormal test result or outcome was identified as follows: (1) an abnormal nonstress test (i.e., nonreactive or with decelerations), (2) sonographic evidence of oligohydramnios (largest pocket of fluid <2 cm in depth) confirmed at amniotomy, (3) moderate or thick meconium, (4) intrapartum fetal distress (defined as repetitive late decelerations or variable decelerations with decreased baseline variability supported by an acidotic fetal scalp pH or umbilical cord gases), and (5) Apgar score of <7 at 5 minutes.

The diagnosis of postmaturity syndrome in the neonate was made if a physical examination showed a loss of subcutaneous fat, long fingernails, and desquamation of the skin. The sample size of the study population was calculated to detect a true difference in the mean peak systolic to end-diastolic ratio of 0.20, risking only a 5% chance of failing to reject the null hypothesis of no difference. Statistical analysis was performed with the Student *t* test.

Results

The mean gestational age at delivery was 42.4 ± 0.4 (±SD) weeks (range 42 to 43.7 weeks) with a mean birth weight of 3.69 ± 0.46 kg. The cesarean section rate was 33% and no perinatal deaths occurred.

The mean peak systolic to end-diastolic ratios for those neonates who were entirely normal, those with signs of postmaturity, and those with abnormal test results or outcome are shown in Table I.

Twenty-one of 46 infants had no abnormal test result, outcome, or signs of postmaturity. Their mean peak systolic to end-diastolic ratio was 2.07 ± 0.20. Nine neonates had a physical examination showing signs of postmaturity; their mean peak systolic to end-diastolic ratio was 2.07 ± 0.26. The difference between the mean peak systolic to end-diastolic ratios of these two groups is not significant.

Seven neonates had an abnormal nonstress test, three had oligohydramnios, 13 had meconium, four had intrapartum fetal distress, and one had a 5-minute Apgar score of <7. The difference between their mean peak systolic to end-diastolic ratios and the normal group is not significant.

Twenty neonates had one or more abnormal test results or outcomes. Their mean peak systolic to end-diastolic ratio was 2.05 ± 0.32, which is also not significantly different from the normal group.

Comment

This study prospectively evaluated the usefulness of umbilical artery flow velocimetry for identifying postdate fetuses at risk. Comparisons of the peak systolic to end-diastolic ratios for neonates with and without the complications associated with postdatism showed no significant differences. All fetuses with abnormal nonstress tests, oligohydramnios, meconium, fetal distress, and the postmaturity syndrome had peak systolic to end-diastolic ratios within the normal range. Consequently, umbilical artery flow velocimetry is unlikely to be useful for routine assessment of fetal status in prolonged pregnancies. These results do not support the conclusions reached by Rightmire et al.,[9] who have recently studied 35 postdate patients and reported abnormal umbilical artery waveforms on fetuses with other than an "acceptable" outcome.

Placental insufficiency describes a process that results in impaired fetal health. The underlying pathology, however, is still poorly understood. Umbilical artery flow velocimetry has helped clarify placental insufficiency associated with intrauterine growth retardation and maternal hypertension. Trudinger et al.[5] have reported elevated peak systolic to end-diastolic ratios in

32 of 43 growth-retarded fetuses. Fleischer et al.[4] have shown that a peak systolic to end-diastolic ratio >3 had a sensitivity of 78% and a specificity of 85% in identifying the small for gestational age fetus. Giles et al.[10] were able to correlate abnormal umbilical artery waveforms with microvascular lesions in the placenta characterized by obliteration of the small muscular arteries in tertiary stem villi. Their findings help explain the increased intervillous resistance and thus the altered fetoplacental circulation found in growth-retarded fetuses.

Umbilical artery flow velocimetry is unlikely to add to the understanding of placental insufficiency associated with postmaturity. In the present study, even the most malnourished, postmature fetus had normal placental blood flow resistance, as evidenced by normal umbilical artery velocity waveforms. Other possible explanations for the underlying pathophysiology resulting in postmaturity need to be explored. There may be changes within the placenta that do not result in increased resistance to blood flow. The fetus may become less efficient in using the nutrients it receives through the placenta. It is also possible that nutrient inflow to the placenta is compromised in postmature pregnancies. From this study, we can only conclude that increased placental resistance, as reflected by abnormal umbilical artery velocity waveforms, does not seem to play a role in the pathogenesis of postmaturity.

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Growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels compared in premature, small, average birth weight, and large infants

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We have investigated the relationship between growth hormone, somatomedin C, nonsuppressible insulin-like activity, weight, gestational age, and 1-minute Apgar score in newborn infants. The 153 infants were categorized as small for gestational age (n = 19), average for gestational age (n = 59), large for gestational age (n = 60), and premature (gestational age at birth, 36 weeks or less (n = 15). Our study showed that (1) growth hormone levels were elevated in premature infants and correlated with Apgar scores and birth weights; (2) somatomedin C and nonsuppressible insulin-like activity levels were significantly lower in premature than in term infants; and (3) the birth weight of all infants studied had a significant overall effect on both somatomedin C and nonsuppressible insulin-like activity levels, suggesting that these factors may be involved in fetal growth. However, because in small for gestational age infants somatomedin C and nonsuppressible insulin-like activity werre similar to levels in average for gestational age infants, it is suggested that other factors may inhibit fetal growth. (AM J OBSTET GYNECOL 1987;157:1524-8.)

Key words: Growth hormone, somatomedin C, nonsuppressible insulin-like activity, fetus

Despite the impressive progress made during the past 2 decades in understanding how hormones and growth factors regulate postnatal growth, the mechanism responsible for fetal growth in utero is poorly understood. The isolation and description of the sequence of insulin-like growth factors I and II from nonsuppressible insulin-like activity by Rinderknecht and Humbel[1] in 1976 shed some light on this important subject. Underwood and D'Ercole[2] purified and separated somatomedin C from adult human plasma and found that its structure was similar to that of insulin-like growth factor I. The isolation of growth factors I and II, also known as somatomedins, was preceded by the description of "typical" and "atypical" insulin-like activity by Samaan et al.[3] and were later called "suppressible" and "nonsuppressible" by Froesch et al.[4] Samaan et al.[5] had strong evidence that nonsuppressible or "atypical" insulin-like activity was formed after regular insulin was infused into the liver. Regular insulin ("typical insulin-like activity), or insulin extracted from the pancreas, was infused into the liver and the nonsuppressible insulin-like activity level was low in patients with liver cirrhosis and after a patient received a portocaval shunt.[6] It was also found that nonsuppressible insulin-like activity, as measured by the fat-pad assay,[7] or somatomedins, as measured by the costal-cartilage assay,[8] was low in both insulin-requiring diabetic patients and thin diabetic adults. Several investigators have considered the possibility that insulin might augment fetal growth by stimulating somatomedin production.[9, 10]

It has also been reported that growth hormone appears to be of minimal importance as a stimulator of fetal growth, since anencephaly and congenital absence of the pituitary gland are associated with normal-size infants, and fetal rabbits, rats, and monkeys that have been hypophysectomized maintain a normal rate of growth in utero.[11, 12] However, it has been shown that the growth hormone may augment the action of the insulin on the liver to form somatomedin.[13]

It has also been found that premature children have high levels of growth hormone at birth, but a correlation between the growth hormone in infants and the degree of stress (Apgar) has not been found.

We undertook this study (1) to determine the rela-

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Table I. Growth hormone, somatomedin C, and nonsuppressible insulin-like activity in premature, SGA, average for gestational age, and LGA infants

Category (n)	*GH (ng/ml)*	*SMC (U/ml)*	*NSILA (μU/ml)*
Premature (15)	23 ± 11	0.46 ± 0.3	130 ± 63
SGA (19)	17.3 ± 12	0.91 ± 0.62	220 ± 102
AGA (59)	13.7 ± 8.5	0.82 ± 0.7	179 ± 113
LGA (60)	13.7 ± 12	1.1 ± 0.8	214 ± 102

AGA = Average for gestational age; GH = growth hormone; SMC = somatomedin C: NSILA = nonsuppressible insulin-like activity.

tionship between growth hormone and the degree of stress at birth as suggested by 1-minute Apgar scores and the length of gestation and (2) to compare nonsuppressible insulin-like activity, somatomedin C, and growth hormone levels in infants who were small for gestational age (SGA) with levels found in premature, large, and normal weight infants to determine the relationship of these hormones to the weight of newborn infants.

Material and methods

Umbilical cord blood was obtained in nonheparinized tubes from the placental side that contained both arterial and venous blood. The serum was separated and kept at −20° C until the time of assay. The blood was obtained from the umbilical cords of 60 large for gestational age (LGA) infants, 19 SGA infants, 59 average for gestational age infants, and 15 premature infants. Categories were based on data provided by Williams et al.[14] in a study conducted in California that used 2,288,806 livebirths. Infants who had reached 37 weeks' gestation and whose weight was in the tenth percentile or less were considered SGA. Those whose weight was in the 90th percentile or more were considered LGA, and those with weights between the 11th and 89th percentiles were considered average for gestational age. An infant was considered premature if the gestational age at birth was 36 weeks or less. The study was conducted with the approval of the Human Protection Committee of the University of Texas Health Science Center and Medical School.

Immunoreactive growth hormone was measured in 0.1 ml serum samples in duplicate by the method described by Samaan et al.[15] The addition of 1000 to 20,000 ng/ml of human placental lactogen in the assay tubes showed no cross-reaction with the growth hormone antibody used. Immunoreactive somatomedin C was measured in unextracted serum by the method of Furlanetto et al.[16] Using these immunoreactive methods, the intraassay variation was <3% and the interassay variation was <10%. The nonsuppressible "atypical" insulin-like activity was measured and transformed into units, which used the fat-pad assay, by the method of Samaan et al.[3] in quadruplicate aliquots of 0.25 ml serum. A reference serum sample (control) was included in each assay. The assay is sensitive to 4 μU insulin. The interassay and the intraassay variation coefficient of variation on different days was <10%.[3]

Table II. A summary of one-way analysis of variance for growth hormone, somatomedin C, and nonsuppressible insulin-like activity in SGA, average for gestational age, LGA, and premature neonates

	Growth hormone	*Somatomedin C*	*Nonsuppressible insulin-like activity*
F ratio	3.00	3.63	2.82
p value	<0.05	<0.05	<0.05

To make an overall comparison that tells whether there is a significant difference between the means of groups, one-way analysis of variance that uses the F test was done to determine the overall effect of birth weight on growth hormone, somatomedin C, and nonsuppressible insulin-like activity hormone levels. A multiple comparison procedure, the Tukey test, was done to locate the specific mean differences. The mean ± standard deviation and *t* test were used to compare the hormone levels between premature and term neonates. Pearson's correlation coefficient was used to analyze the correlation between hormone levels and one-minute Apgar scores. The Apgar scores recorded in the chart at the time of birth were used. The distribution of patients among groups was analyzed according to χ^2.

Results

We initially attempted to present the hormonal data by matching the infants by exact gestational age and then by analyzing the mean hormonal values to determine the relationship between weight and the values of growth hormone, somatomedin C, and nonsuppressible insulin-like activity. We believed this type of analysis was important because the distribution of subjects was significantly different with respect to premature, SGA, average for gestational age, and LGA infants. There were more LGA neonates in the 40 to 43 weeks' category than in the less than 37 weeks category ($p < 0.001$). However, too few infants were in each gestational week for gestational matching to be statistically meaningful. Therefore the data were combined as described and defined by the aforementioned method: premature, SGA, average for gestational age, and LGA.

The levels of growth hormone, somatomedin C, and nonsuppressible insulin-like activity in the SGA, pre-

Table III. Tukey test results on growth hormone, somatomedin C, and nonsuppressible insulin-like activity in premature, SGA, average for gestational age, and LGA infants

Comparison	*Mean difference*	*Standard error*	*q*	*p*
GH				
Premature vs LGA	9.2	2.40	3.83	<0.05
Premature vs AGA	9.2	2.41	3.82	<0.05
Premature vs SGA	5.6	2.86	1.96	>0.1
SGA vs LGA	3.6	2.11	1.71	>0.1
SGA vs AGA				>0.1
AGA vs LGA				>0.1
SMC				
LGA vs premature	0.64	0.146	4.38	<0.05
LGA vs AGA	0.27	0.095	2.84	>0.1
LGA vs SGA				>0.1
SGA vs premature	0.45	0.18	2.5	>0.1
SGA vs AGA				>0.1
AGA vs premature	0.37	0.146	2.53	>0.1
NSILA				
SGA vs premature	90.05	5.37	16.77	<0.05
SGA vs AGA	41.11	23.46	1.75	>0.1
SGA vs LGA				>0.1
LGA vs premature	83.96	22.56	3.72	<0.05
LGA vs AGA	35.02	16.75	2.09	>0.1
AGA vs premature	48.94	21.62	2.26	>0.1

GH = Growth hormone; AGA = average for gestational age; SMC = somatomedin C; NSILA = nonsuppressible insulin-like activity.

Table IV. Mean ± SD, growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels in premature and term neonates

	Growth hormone	*Somatomedin C*	*Nonsuppressible insulin-like activity*
Premature (n = 15)	23 ± 11	0.46 ± 0.34	130 ± 63
Term	14.2 ± 10.6	1.02 ± 1.0	198 ± 108
	$p < 0.001$	$p < 0.05$	$p < 0.02$

Table V. Pearson's correlation coefficient in 153 infants between growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels and 1-minute Apgar scores

	Growth hormone	*Somatomedin C*	*Nonsuppressible insulin-like activity*
r value	−0.262	0.06	0.107
p value	<0.01	>0.1	>0.1

mature, average for gestational age, and LGA infants are presented in Table I. When serum growth hormone levels were subjected to one-way analysis of the variance, it was found that the infant's birth weight has an overall effect on growth hormone levels (Table II). The Tukey test was performed to locate the specific mean differences among the four groups (Table III). It was found that the growth hormone of premature infants was significantly higher than levels in LGA and average for gestational age infants ($p < 0.05$). When growth hormone levels were compared according to premature and term neonates, the growth hormone was significantly higher in premature neonates (23 ± 11 versus 14.2 ± 10.6, $p < 0.001$) (Table IV).

Somatomedin C levels were subjected to one-way analysis of variance. It was found that the birth weight of an infant has an overall effect on somatomedin C levels (Table II). According to the Tukey test, somatomedin C was significantly lower in premature infants compared with LGA infants ($p < 0.05$, Table III). When somatomedin C levels were compared in premature and term neonates, somatomedin C was significantly lower in the premature group (0.46 ± 0.34 versus 1.02 ± 1.0, $p < 0.01$) (Table IV).

When nonsuppressible insulin-like activity was subjected to one-way analysis of the variance, it was found that birth weight had an overall effect on nonsuppressible insulin-like activity levels (Table II). According to the Tukey test, nonsuppressible insulin-like activity was significantly less in premature infants compared with SGA and LGA infants ($p < 0.05$, Table III). When nonsuppressible insulin-like activity levels were compared according to premature and term neonates, premature infants had a significantly lower level of nonsuppressible insulin-like activity (130 ± 63 versus 198 ± 108, $p < 0.02$) (Table IV).

To determine if there was a correlation between growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels and one-minute Apgar scores, a Pearson's correlation coefficient was calculated for all 153 infants (Table V). There was a significant negative correlation between one-minute Apgar scores and growth hormone levels in infants. There was no significant correlation between somatomedin C or nonsuppressible insulin-like activity and the one-minute Apgar score (Table V).

Comment

Various investigators have shown that growth hormone has minimal importance as a stimulator of fetal growth.[12, 13] In our studies, the growth hormone level was significantly higher in premature infants than in those of normal birth weight or in large infants, thus supporting the notion that growth hormone may not play an important part in fetal growth. We also found paradoxic relationships between the levels of growth hormone, somatomedin C, and nonsuppressible insulin-like activity in premature children compared with levels in SGA, average for gestational age, and LGA infants. Our data suggest that hormonal levels of the growth hormone, somatomedin C, and nonsuppressible insulin-like activity in neonates are a reflection of gestational age and birth weight. When term infants were categorized according to birth weight, there was an overall effect on growth hormone, somatomedin C, and nonsuppressible insulin-like activity. When these infants were compared with premature infants, there was a difference in these hormonal values. We also found that growth hormone levels correlated negatively with Apgar scores.

It has been suggested that insulin may be the main hormone responsible for intrauterine fetal growth either by its direct action or stimulation of somatomedin formation.[10, 11] This assumption was based on the observation that the administration of insulin to fetal rabbits raises plasma somatomedin activity and endogenous cartilage growth activity.[10] Similarly, Spencer et al.[11] observed that pig fetuses that were made chronically hyperinsulinemic between 90 to 104 days of gestation had significant elevations in plasma somatomedin bioactivity.

One of the early observations on somatomedins was their stimulatory effect on deoxyribonucleic acid synthesis and cell multiplication and the incorporation of inorganic sulfate into cartilage.[15, 17] These observations have been confirmed with pure insulin-like growth factor I and II.[17] It has been shown in long-term in vivo studies that insulin-like growth factor I is capable of stimulating growth indexes and body weight in the absence of growth hormone. The corresponding effect of insulin-like growth factor II is much less pronounced, and the true physiologic role of insulin-like growth factor II remains to be elucidated.[17]

We have previously shown significantly higher levels of both somatomedin C and nonsuppressible insulin-like activity in average for gestational age and LGA infants compared with premature infants,[19] but the levels of these two hormones and their relationship with the weights of SGA infants has not been studied.[18]

In the present study, we found that levels of somatomedin C and nonsuppressible insulin-like activity were affected by birth weight. However, since the levels of somatomedin C and nonsuppressible insulin-like activity were similar between the SGA and average for gestational age infants, we conclude that other factors may also be involved in inhibiting fetal growth of SGA infants. Our results also showed that growth hormone levels in neonates correlate with body weight. Growth hormone levels were highest in premature neonates and Apgar scores in this group were lowest. Although this may reflect the gestational age of the newborn infant, stress cannot be excluded from consideration.

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Seminal plasma proteins of fertile and infertile men analyzed by two-dimensional electrophoresis

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Sperm-free seminal plasma from seminal fluid ejaculate of fertile and infertile men obtained in the presence and absence of aprotinin (500 kallikrein inhibitor units per milliliter) was analyzed by two-dimensional electrophoresis followed by silver staining. To evaluate postliquefaction proteolytic breakdown of seminal plasma proteins, protease inhibitors were added to the semen at 15, 30, and 60 minutes after ejaculation. Most seminal plasma proteins in normospermic men (n = 4) had molecular weights of 30,000 to 70,000 and were similar to those present in serum. The major non–serum protein in seminal plasma of all men was a basic product with an approximate molecular weight of 40,000. A group of proteins (molecular weights = 20,000 to 23,000) in seminal plasma analyzed immediately after liquefaction was detected in oligospermic men (n = 4) but not in normospermic men (n = 4) or azoospermic men (n = 4). When protease inhibitor was added to normospermic semen at ≥15 minutes after liquefaction, these proteins (molecular weight = 20,000 to 23,000) and another group of proteins (molecular weights = 40,000 to 43,000) were readily identifiable but were further enhanced in the absence of protease inhibitors. These findings suggest that oligospermic men may have accelerated proteolysis of sperm or seminal plasma proteins that may contribute to subfertility. (AM J OBSTET GYNECOL 1987;157:1528-33.)

Key words: Seminal plasma proteins, oligospermia, azoospermia, two-dimensional electrophoresis, subfertility

Semen analysis continues to be a primary laboratory method of evaluating male fertility despite its poor predictive value except for those men with severe abnormalities. More recently, the hamster zona-free oocyte penetration test and in vitro cervical mucus penetration test have added new dimensions to the evaluation of male gametes. However, these tests also are not consistently reliable. Human seminal plasma has 18 to 55 gm protein per liter[1, 2] with 40% to 60% derived from the seminal vesicles, 35% to 40% from the prostate, and the remainder from other accessory glands. A fluid such as human seminal plasma with its complex composition and the protein contribution from the male reproductive tract is likely to have an effect on sperm survival and quality. A few limited studies have examined the electrophoretic patterns of the proteins in human seminal plasma,[3, 4] but interpretation of the data

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is difficult since protease inhibitors were not added to the semen examined. Basic proteins in human seminal plasma have been shown to undergo rapid proteolysis 15 minutes after ejaculation.[5] To determine if there are differences in the protein profile of seminal plasma of fertile and subfertile men, we performed a study with two-dimensional electrophoresis to map the acidic and basic seminal plasma proteins with and without the addition of the protease inhibitor aprotinin to the semen of normal, oligospermic, and azoospermic men. This article reports the findings of our study.

Material and methods

Electrophoresis supplies. *Bis*-acrylamide and N,N,N′,N′-tetramethylethylenediamine were obtained from Biorad Laboratories, Richmond, Calif., and acrylamide was a product of Serva Biochemicals, Heidelberg, Germany; N,N′-diethyl-tartardiamide was purchased from National Diagnostics, Somerville, N.J., and ampholines were from LKB, Uppsala, Sweden. Coomassie blue R-250, sodium dodecyl sulfate, urea, dithiothreitol, protein standards, 2β-mercaptoethanol, agarose, and all other inorganic chemicals of reagent grade or better were products of Sigma Chemical Co., St. Louis, Mo. The protease inhibitor aprotinin was also purchased from Sigma Chemical Co.

Subjects and patients. Male volunteers 18 to 28 years of age were recruited from our Infertility Clinic. All subjects gave consent to provide semen samples. Normal men were defined as those who had fathered children and had two or more normal semen analyses at intervals of at least a month (sperm count >20 million/ml, >50% motility, and <40% sperm with abnormal morphologic features). Oligospermic men were those who had a history of infertility, three or more semen analyses ⩾1 month apart with a sperm count of <20 million/ml, >50% motility, and >50% sperm with normal morphologic features. Azoospermic men were those who had a history of infertility and no sperm in the ejaculate but who had fructose in the seminal plasma on at least two semen analyses ⩾1 month apart. Four normospermic, four oligospermic, and four azoospermic men were studied.

Collection of semen samples. The subjects in the study were asked to abstain from sexual activity for at least 36 hours before providing a semen sample. Ejaculates were collected by masturbation either into sterile containers without protease inhibitors or into containers that had previously been coated with 1750 kallikrein inhibitor units of aprotinin. After liquefaction, a small aliquot of each of the ejaculates was set aside for routine semen analysis. The semen samples were held at room temperature for 0, 15, 30, or 60 minutes after liquefaction. An aliquot of 250 to 500 μl (depending on the volume of the ejaculate) was removed from each ejaculate and the spermatozoa were separated from the seminal plasma by centrifugation in a microcentrifuge (10,000 g × 10 minutes). Ejaculates obtained in the absence of any aprotinin were further divided into two aliquots. One aliquot was kept at room temperature for 15, 30, or 60 minutes while aprotinin (500 kallikrein inhibitor units/ml) was added to the other at similar time intervals, then mixed in a vortex and centrifuged. The seminal plasma obtained at each time point for each treatment was separated from the sperm after centrifugation and stored at −70° C. The protein concentration in the seminal plasma obtained at each time point was determined by the method of Lowry et al.[6]

Two-dimensional polyacrylamide gel electrophoresis. Samples of seminal plasma (300 μg protein) were dissolved in 75 μl of 5 mmol/L potassium carbonate containing 9.3 mol/L urea, 2% (vol/vol) NP-40, and 0.5% (w/vol) dithiothreitol for analysis by two-dimensional polyacrylamide gel electrophoresis. The samples were analyzed by isoelectric focusing according to the method of O'Farrell[7] as modified by Horst et al.[8]

The proteins (pI < 8.0) were analyzed by isoelectric focusing in the first dimension in 4% (w/vol) acrylamide gels containing ampholines (3.5 to 10, 5 to 7, and 9 to 11, 50:35:15 by volume, respectively).

After isoelectric focusing was performed, the samples were analyzed in the second dimension by sodium dodecyl sulfate gel electrophoresis.[9] The equilibrated electrofocused gels were overlaid on 10% (w/vol) acrylamide slab gels and electrophoresis was performed toward the anode. The slabs were fixed in two changes of ethanol, acetic acid, and water (40:7:53 by volume, respectively), stained with 0.125% Coomassie blue R-250, destained, and photographed. The gels of seminal plasma proteins were then stained with silver.[10]

Results

In the seminal specimens from all male subjects studied, the major non–serum protein present in seminal plasma was a basic protein with an approximate molecular weight of 40,000. Most of the seminal plasma proteins present in normospermic men had molecular weights of 30,000 to 70,000 and were similar to those present in the serum (Fig. 1).

In oligospermic men, a group of proteins with molecular weights of 20,000 to 23,000 was present in the seminal plasma immediately after liquefaction. However, these proteins were not detected in seminal plasma of either normospermic or azoospermic men at the same time point (Fig. 1).

Fig. 2 shows the silver-stained two-dimensional electrophoretic gels of seminal plasma from normal men and the effect of proteolysis at room temperature on the appearance of the group of proteins (molecular weights 20,000 to 23,000). When the protease inhibitor

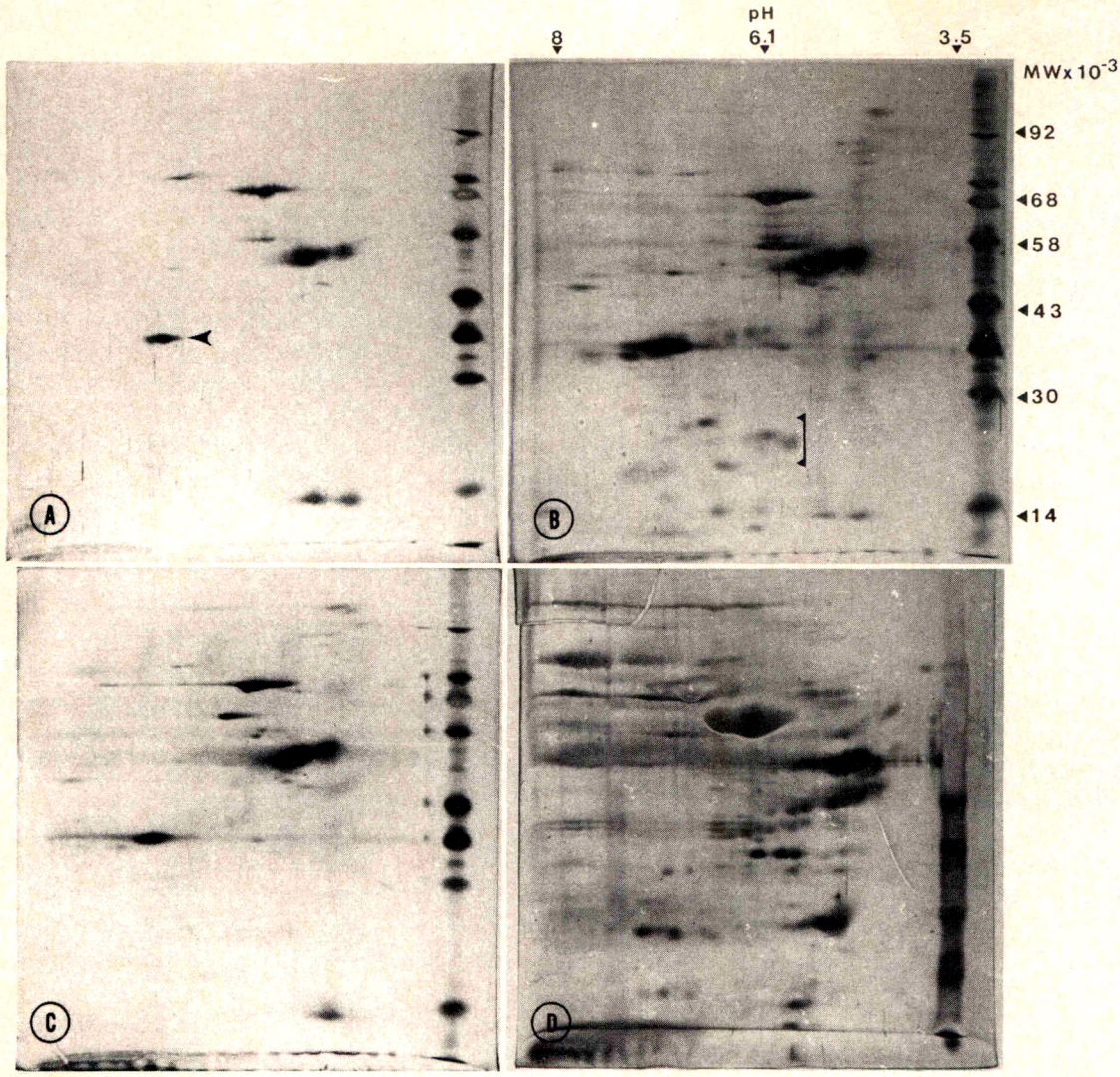


Fig. 1. Silver-stained two-dimensional electrophoretic gels of seminal plasma from normospermic *(A)*, oligospermic *(B)*, and azoospermic *(C)* men and of human plasma *(D)*. Seminal fluid ejaculates were collected in the presence of protease inhibitor. A group of proteins with molecular weights of 20,000 to 23,000 ( ) is present in seminal plasma from oligospermic men *(B)* but is absent in normospermic *(A)* and azoospermic men *(C)*. The *arrow* indicates the basic protein present in seminal plasma but not in serum.

aprotinin was not added to the ejaculate after liquefaction, the group of proteins (molecular weights 20,000 to 23,000) that was previously detected only in seminal plasma of oligospermic men (Fig. 1, *B*) appeared and increased in intensity over time. In addition, another group of proteins (molecular weights 40,000 to 43,000) also appeared in normal seminal fluid with this treatment.

When proteolysis of semen from normospermic men was inhibited by the addition of the protease inhibitor aprotinin at 15, 30, and 60 minutes after liquefaction, the two groups of proteins (molecular weights 20,000 to 23,000) that were present in normal semen after proteolysis were then also detectable by two-dimensional electrophoresis (Fig. 3). However, compared with Fig. 2, the intensity of these proteins was significantly diminished over time (Fig. 3).

Comment

Previous limited studies on seminal plasma proteins have examined only the semen of normal men.[3-5] Using two-dimensional gel electrophoresis, we found that the major non–serum protein present in the seminal plasma of both fertile and subfertile men was a basic protein with a molecular weight of 40,000. This is similar to the earlier reports of semen from normal men.[3-5]

Of greater significance, however, was our finding that

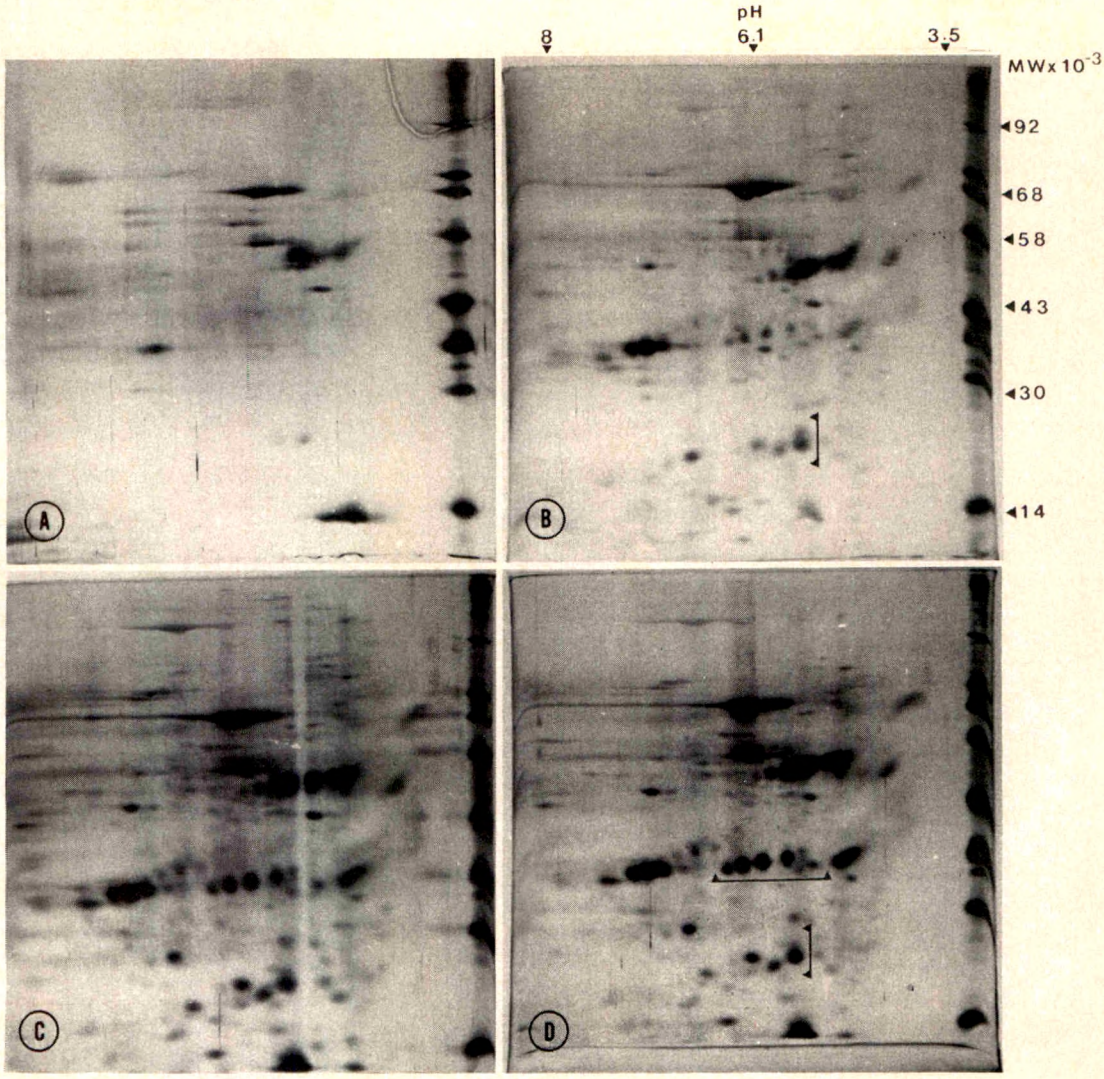


Fig. 2. Silver-stained gels after two-dimensional electrophoresis of seminal plasma from normospermic men. Ejaculates were collected in the absence of the protease inhibitor aprotinin and kept at room temperature for 0 *(A)*, 15 *(B)*, 30 *(C)*, and 60 *(D)* minutes after liquefaction. The group of proteins with molecular weights of 20,000 to 23,000 (⌈⌉) that was present in Fig. 1, *B*, appeared and increased in intensity over time after liquefaction. In addition, another group of proteins with molecular weights of 40,000 to 43,000 (▲—▲) also appeared and increased in intensity over time.

a group of proteins with molecular weights of 20,000 to 23,000 is present in the seminal plasma of oligospermic men if analysis is done immediately after liquefaction but not in the seminal plasma of normal or azoospermic men. Nevertheless, when a protease inhibitor such as aprotinin is added to normal semen 15 or more minutes after liquefaction, these proteins (molecular weights = 20,000 to 23,000) are readily identified. In addition, another group of proteins with molecular weights of 40,000 to 43,000 also became identifiable in normal seminal fluid treated with the protease inhibitor. Furthermore, the identification of these two groups of proteins (molecular weights = 20,000 to 23,000 and 40,000 to 43,000) was enhanced in the absence of protease inhibitors. Thus, a group of proteins with molecular weights of 20,000 to 23,000 is readily detected in seminal plasma immediately after ejaculation and liquefaction in oligospermic individuals. This group of proteins (molecular weights = 20,000 to 23,000) together with another group (molecular weights = 40,000 to 43,000) are detectable in the semen of normospermic individuals only when proteolytic activity is not suppressed. These proteins could not be identified in the semen of azoospermic men.

The proteins with molecular weights of 20,000 to 23,000 are most likely the products of accelerated pro-

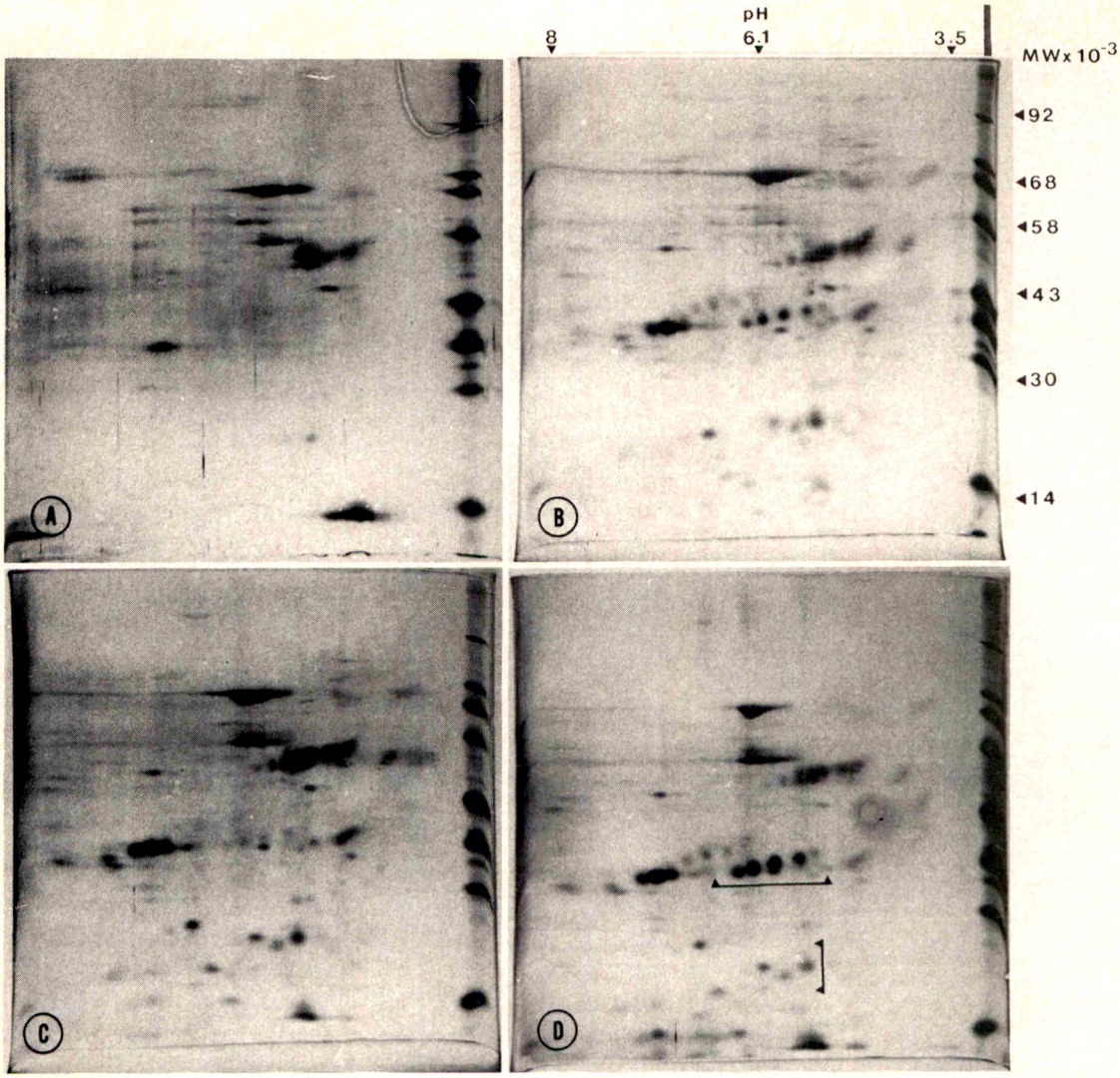


Fig. 3. Silver-stained two-dimensional electrophoretic gels of seminal plasma from normospermic men. The ejaculates were collected in the absence of the protease inhibitor aprotinin that was added at 15, 30, and 60 minutes after liquefaction. The group of proteins with molecular weights of 20,000 to 23,000 ( ) that was found in oligospermic men in Fig. 1, *B*, and under the conditions in Fig. 2 in normospermic men as well as the other group of proteins with molecular weights of 40,000 to 43,000 (▲—▲) that appeared under the conditions in Fig. 2 is now present but *diminished in intensity* over time as compared with Fig. 2.

teolysis and originate from spermatozoa that undergo proteolytic degradation. The absence of these proteins in the seminal plasma of azoospermic individuals with or without varying degrees of proteolytic inhibition suggests the spermatozoa as the source of these proteins. The rapid appearance and easy detection of these proteins in seminal plasma of oligospermic men combined with enhancement of their detection in normal seminal plasma when proteolysis is not inhibited strongly support the role of accelerated proteolysis for the generation of these proteins. Therefore, the seminal fluid of oligospermic men has apparently accelerated proteolytic activity. What is less clear, however, is whether such apparently accelerated proteolytic activity is due to increased levels of proteolytic enzyme(s) activity and/or increased susceptibility of spermatozoa to proteolytic degradation in the semen of oligospermic men. Our hypothesis and explanation can and should be further explored in investigations on previously fertile men who have undergone vasectomy, but this was not within the original scope of this study.

While previous studies have examined seminal plasma from normal men and as many as 200 protein bands were found on the two-dimensional electropho-

resis,[3-5] similar analyses have not been reported for oligospermic and azoospermic men. Nonetheless, interpretation of the data from these studies[3-5] was complicated by the fact that protease inhibitors were not added to the semen collected. The basic proteins in seminal plasma undergo rapid proteolysis 15 or more minutes after ejaculation.[5] Our findings show that proteins of 20,000 to 23,000, which are products of accelerated proteolysis in oligospermic men, also appear in normospermic seminal plasma, at a slower but normal rate of proteolysis. Furthermore, another group of proteins with molecular weights of 40,000 to 43,000 is also a product of the proteolysis of normal semen but does not show up readily in oligospermic seminal plasma. Therefore, there is a need to examine proteolysis and its inhibition and the possible contribution by sperm degradation to the appearance of some proteins in seminal plasma by studying seminal plasma of individuals with normospermia, oligospermia, and azoospermia as well as the effect of protease inhibitors on the presence and detection of any proteins.

It is not clear whether the proteins (molecular weights = 20,000 to 23,000) that we found in the seminal plasma of oligospermic men reflect subfertility or give rise to infertility. If proteolysis of spermatozoa is accelerated in the ejaculates of oligospermic men, the number of spermatozoa available for ascent into the female reproductive tract could be reduced. On the other hand, such accelerated proteolysis may eliminate abnormal or nonviable spermatozoa as a means of ensuring selection of the more optimal spermatozoa, since proteolysis also occurs in normal semen but at a slower rate. Further studies are necessary to clarify these mechanisms.

In conclusion, we have found accelerated proteolysis in the semen of oligospermic men and it is readily demonstrable early after ejaculation and liquefaction by two-dimensional gel electrophoresis. This derangement could be a contributory factor to the infertility of oligospermic men.

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The effect of insulin on aromatase activity in isolated human endometrial glands and stroma

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Hyperinsulinemic states have been associated with an increased incidence of estrogen-dependent endometrial neoplasia. To study the effect of insulin on the ability of endometrium to aromatize androgens to estrogens, late proliferative endometrium was obtained from normally cycling women at the time of indicated surgery, separated into component glands and stroma, and grown to confluence. Separated gland and stromal cultures were incubated in triplicate with increasing insulin concentrations and epidermal growth factor. Aromatase activity was assayed by the production of tritiated water from tritium-labeled androstenedione. The activity was noted to increase proportionally with increasing concentrations of insulin >10 U/ml, and the effect was specific. These data suggest the following conclusions: (1) Insulin stimulates aromatase activity in both endometrial glands and stroma; (2) hyperinsulinemia may predispose to endometrial neoplasia by enhancing endogenous endometrial estrogen production. (Am J Obstet Gynecol 1987;157:1534-9.)

Key words: Aromatase, endometrium, epidermal growth factor, insulin

Endometrial neoplasia has been linked epidemiologically to both long-term unopposed estrogen exposure and hyperinsulinemia as independent risk factors.[1, 2] The mechanisms linking estrogens and endometrial neoplasia have been studied extensively, whereas the association between insulin and endometrial neoplasia remains largely unexplored. Several recent lines of evidence, when taken together, suggest a mechanism for this association.

Tseng et al.[3] demonstrated the ability of endometrium to convert androgens to estrogens by aromatization, which suggests a potential role for endogenously produced estrogen in local stimulation of endometrial proliferation. Garzo and Dorrington[4] described augmentation of follicle-stimulating hormone (FSH)–induced aromatization in granulosa cells by insulin. Sheets et al.[5] demonstrated the existence of endometrial insulin receptors, which documents binding of insulin by endometrial plasma membranes. A possible role for endogenous endometrial stimulation by insulin-induced augmentation of endometrial aromatization was thus postulated.

This study was undertaken to test the hypothesis that insulin modulates aromatase activity in human proliferative endometrium.

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Material and methods

Endometrium was obtained by curettage from normally cycling parous women undergoing elective laparoscopic tubal sterilization on days 9 through 12 of the menstrual cycle. All women were 25 to 35 years of age, were not receiving hormones, were euthyroid, and had no evidence of hepatic dysfunction. This study was approved by the Human Research Committee of the University of Michigan Medical Center.

Endometrial separation and culture. Endometrial specimens were placed in 50 ml of iced, sterile, normal saline solution and transported to the laboratory where they were processed immediately. Stromal cells were separated from glands by a modification of the method of Liu and Tseng.[6] The tissue was washed with calcium and magnesium-free Earle's balanced salt solution (Gibco, Grand Island, N.Y.) and minced into 1 mm^3 fragments. Tissue separation was achieved by digesting the fragments (0.2 to 0.5 gm tissue/5 ml) in Earle's balanced salt solution containing collagenase (300 U/ml, Cooper Biomedical, Malburn, Penn.), deoxyribonuclease (1 μg/ml, Cooper Biomedical), bovine serum albumin (3 mg/ml, bovine serum albumin fraction V, Sigma Chemical Co., St. Louis, Mo.), gentamicin (50 μg/ml, Elkins-Sinn, Inc., Cherry Hill, N.J.), and mycostatin (2 U/ml, Gibco) at 37° C in a water bath with constant stirring. Free stromal cells were removed by centrifugation from the collagenase solution at 15-minute intervals during the 2-hour digestion period

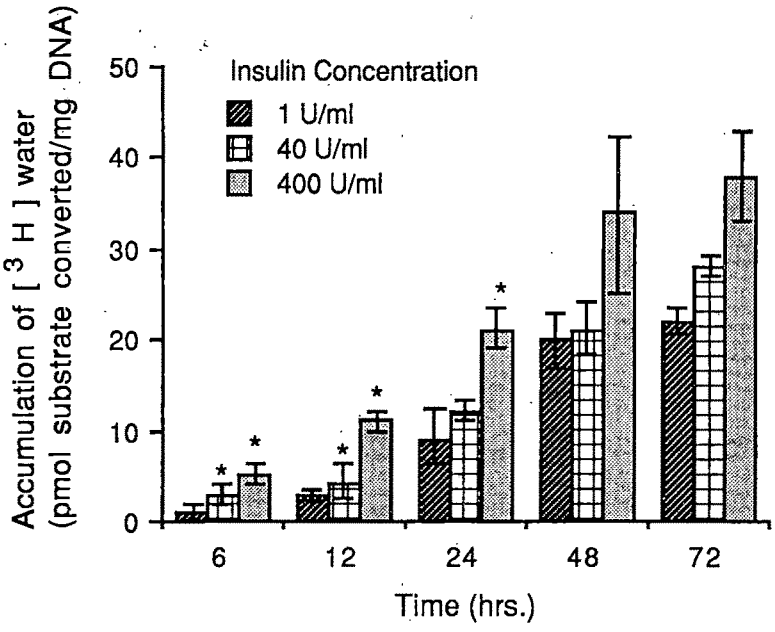


Fig. 1. The effects of time and insulin concentration on aromatase activity in isolated proliferative endometrial stromal cells in confluent monolayer culture. *Bars* represent mean values ± SEM. *DNA* = Deoxyribonucleic acid. * = $p < 0.05$ versus controls.

and resuspended in culture medium A (McCoy's modified 5A medium containing 10% fetal calf serum (Gibco), gentamicin (50 μg/ml, Elkins-Sinn) and mycostatin (2 U/ml, Gibco). Cell viability averaged 70% on the basis of the trypan blue exclusion method. When necessary, erythrocytes were removed by layering the crude stromal cell suspension over Ficoll-Hypaque (Pharmacia, Inc., Piscataway, N.J.) and centrifuging at 700 × g for 15 minutes. After the 2-hour stromal cell separation, the remaining glands were incubated with collagenase for an additional 45 minutes. The stromal and gland cell suspensions were washed three times with medium A and plated into 35 by 10 mm culture dishes (Falcon Plastics, Becton, Dickinson & Co., Oxnard, Calif.) at 2×10^6 cells/2 ml medium A per dish and incubated at 37° C under water-saturated 95% air/5% carbon dioxide. Confluence was reached 3 to 4 days after plating. Morphologic assessment of the separated stromal and gland cell cultures was made by the method described by Liu and Tseng,[6] and a similar 95% purity was found.

Aromatase assay. Aromatase activity was assayed by a modification of the method of Thompson and Siiteri[7] on the basis of the equimolar production of tritiated water during the aromatization of (1β-^{3}H)-androstenedione. Cultures were rinsed with 0.1% bovine serum albumin–McCoy's medium and incubated at 37° C under water-saturated 95% air/5% carbon dioxide with 0.5 μCi (1β-^{3}H)-androstenedione (27.8 Ci/mmol, New England Nuclear, Boston, Mass.). At the completion of each incubation, the culture dishes were placed on ice and the medium was removed and assayed for the incorporation of tritium into tritiated water. In brief, 1 ml of spent media was mixed with trichloroacetic acid (10% final concentration), placed on ice for 15 minutes, and then centrifuged at 3000 × g for 10 minutes. The supernatant was extracted with 3 ml of chloroform. An aliquot of 1 ml of the aqueous layer was added to the charcoal pellet obtained from 1 ml of 5% Norit-A (Fisher Scientific Co., Fairlawn, N.J.), mixed, and placed on ice for 15 minutes. After centrifugation for 15 minutes at 3000 × g, 10 ml Safety-Solve (Research Products International Corp., Mt. Prospect, Ill.) was added to the supernatant, which was then counted in a scintillation counter. Cells were washed, scraped from the dishes, and assayed for deoxyribonucleic acid according to the method of Burton.[8] All cultures were run in triplicate and each assay included triplicate blanks receiving all reagents but no cells. Values obtained for blanks were subtracted from all assay values. This laboratory has previously verified this assay and demonstrated a good correlation between tritiated water produced and total estrogen production.

Statistical analysis was performed by a two-tailed Student's t test and p values were calculated by the least-squares method. Statistical significance was defined as $p < 0.05$.

Results

As a preliminary study to establish the presence of an insulin effect and to determine an optimal time

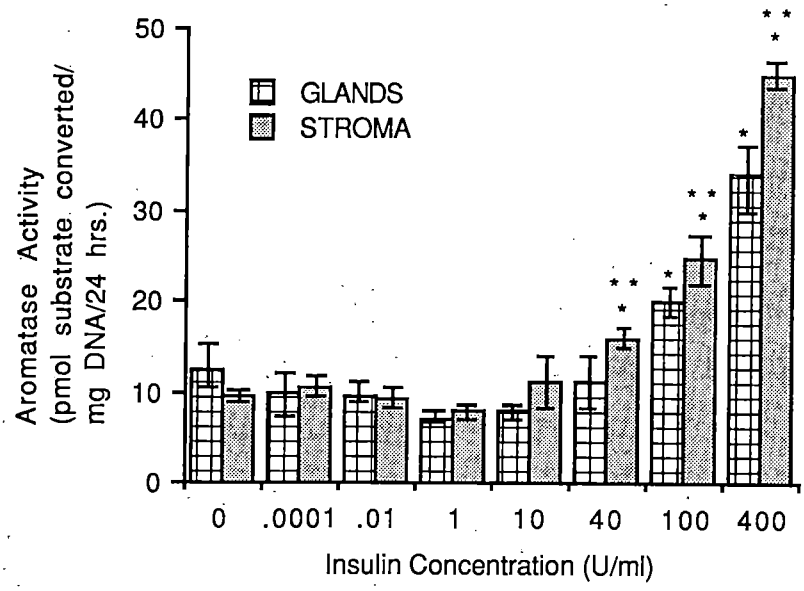


Fig. 2. The effect of insulin concentration on aromatase activity in isolated proliferative endometrial gland and stromal cells in confluent monolayer culture. * = $p < 0.05$ versus controls. ** = $p < 0.05$ versus gland cells.

course for assays, stromal cultures were incubated with 7 nmol/L (1β-^{3}H)-androstenedione and 1, 40, or 400 U/ml insulin for periods of 6, 12, 24, 48, and 72 hours. The endometrial stroma, as depicted in Fig. 1, demonstrated a statistically significant increase in aromatase activity at the highest insulin concentration through 24 hours. The 48- and 72-hour assays demonstrated an apparent increase in aromatase activity, but the standard error was large and statistical significance was not reached. Approximately 25% detachment of cells was noted in the cultures after 24 hours at 400 U/ml insulin, which perhaps accounts for the variability and lack of significance. Therefore, all subsequent assays used a 24-hour incubation period. A statistically significant increase in aromatase activity was also seen in the presence of the 40 U/ml insulin concentration at 6 and 12 hours of incubation.

To determine the effect of insulin concentration on aromatase activity in both isolated glands and stroma, separated gland and stromal cell cultures were incubated with 7 nmol/L androstenedione for 24 hours with increasing concentrations of insulin as depicted in Fig. 2. A statistically significant increase in aromatase activity was noted in the gland cell cultures at all insulin concentrations of ≥100 U/ml and in the stromal cell cultures at all insulin concentrations of ≥40 U/ml. The increase in aromatase activity at each insulin concentration was greater in the stromal cell cultures than in the gland cell cultures. The increase in aromatase activity with increase in insulin concentration was nearly linear in the gland cell cultures and demonstrated some falloff at higher insulin concentrations in the stromal cultures, presumably because of cell detachment.

The kinetics of both the basal and the insulin-induced aromatase activity were studied as summarized in part in Fig. 3. Separated cell cultures were incubated with 10, 110, and 210 nmol/L (1β-^{3}H)-androstenedione and 0, 10, and 100 U/ml insulin. The Michaelis-Menten constant for the reaction of each cell type remained constant at all insulin concentrations, whereas the maximum velocity increased significantly at higher concentrations.

Specificity of the insulin effect was examined by incubating separated cultures with 200 ng/ml epidermal growth factor and parallel cultures with 1, 10, and 100 U/ml insulin. As demonstrated in Fig. 4, epidermal growth factor did not stimulate an increase in aromatase activity, whereas insulin stimulated an increase at both 10 and 100 U/ml. No difference was noted in insulin induction of aromatase activity at any concentration between the two cell types in this experiment.

Comment

The positive correlation between endometrial aromatase activity and insulin concentration noted in this study provides the first evidence for a physiologic link between insulin and endometrial neoplasia. The clinical association between hyperinsulinemia and endometrial neoplasia has been well recognized,[1,2] but the physiologic basis for this association has remained obscure. Unopposed estrogen stimulation of endometrium has been well established as the major risk factor for en-

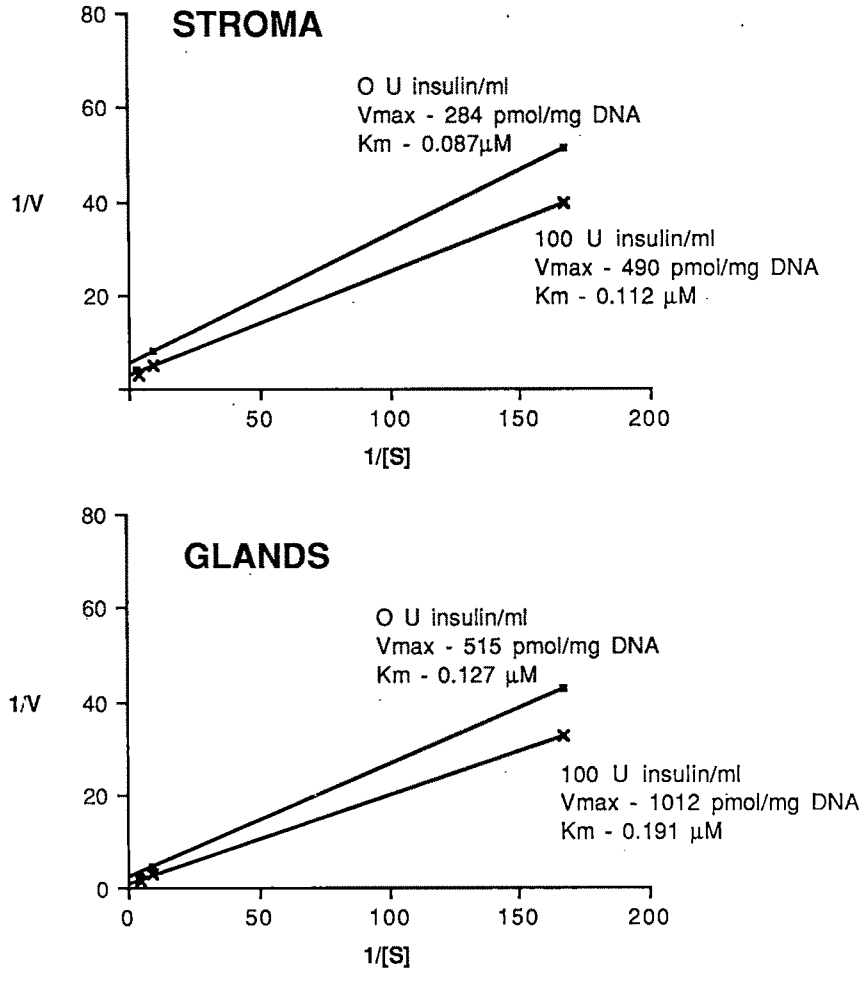


Fig. 3. Enzyme kinetics of basal and insulin-stimulated aromatase activity in isolated endometrial gland and stromal cultures. *Vmax* = Maximum velocity. *Km* = Michaelis-Menten constant.

dometrial neoplasia, and the discovery that endometrium was capable of endogenously producing estrogen[3] has led to speculation on the role of locally produced estrogen in the genesis of endometrial neoplasia. With the findings that endometrium harbored receptors for insulin[5] and that insulin can augment aromatase activity in another reproductive tissue, granulosa cells,[4] the possibility of insulin-induced aromatase activity in endometrium was entertained. This study demonstrates for the first time that insulin can increase aromatase activity in endometrium.

Endometrial aromatase activity has been shown to be influenced by a number of factors. Tseng[9] demonstrated that progesterone markedly increased aromatase activity and that estradiol augmented this progesterone-induced increase in aromatase activity. Estradiol alone did not change aromatase activity. Tseng et al.[10] demonstrated that a number of synthetic progestins increased aromatase activity, with medroxyprogesterone acetate demonstrating the greatest stimulation. In that same study, no increase in aromatase activity was noted with 17α-hydroxyprogesterone, corticosteroids, or androgens.

Most recently, Tseng et al.[11] reported on the effects of relaxin, insulin, and epidermal growth factor on aromatase activity in cultured human endometrial stromal cells. Relaxin alone did not increase aromatase activity but augmented the stimulatory effect of medroxyprogesterone acetate or medroxyprogesterone acetate and estradiol. Insulin, in concentrations of 0.1, 1, and 10 U/ml, did not augment the medroxyprogesterone acetate– and estradiol-induced increase in aromatase activity, but the effect of insulin alone was not reported. Epidermal growth factor (100 ng/ml) alone did not significantly change aromatase activity, a result consistent with the findings of the current study.

Late proliferative endometrium was chosen in this initial study of insulin modulation of aromatase activity since it demonstrates greater activity than secretory endometrium[3] and was felt to be the normal endo-

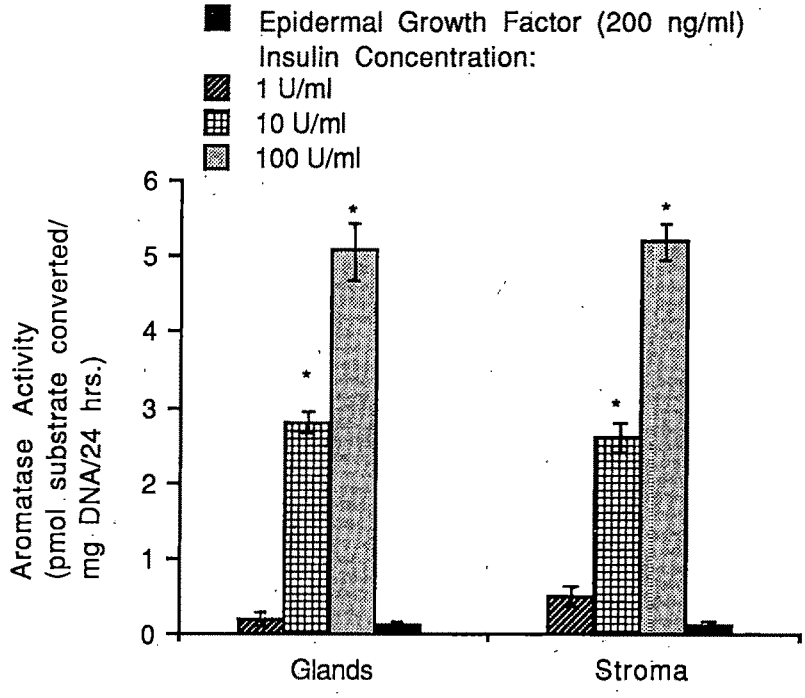


Fig. 4. The effect of insulin and epidermal growth factor on aromatase activity in isolated proliferative endometrial gland and stromal cells in confluent monolayer culture. * = $p < 0.05$ versus controls.

metrium most closely resembling endometrium undergoing chronic unopposed estrogen stimulation. Separated gland and stromal cell cultures were used to determine if a difference in the aromatase activity in the two principal tissues in endometrium could be found. Since most endometrial carcinomas are adenocarcinomas of epithelial origin, the possibility of an enhanced response to insulin in glandular tissue with greater estrogen production and more proliferation was proposed. Such an enhanced response in gland cells was not seen, with a similar or slightly decreased stimulation actually noted. As with any separated cell culture system, purity of the culture was a concern. The characteristic morphologic features of the two cell types in culture permitted the accurate determination of purity on morphologic criteria, as described by Satyaswaroop et al.[12] and by Liu and Tseng.[6]

The assay to determine aromatase activity was initially described and verified by Thompson and Siiteri[7] and subsequently verified by a number of authors, including Tseng et al.[10] The concentrations of insulin required to demonstrate an increase in aromatase activity were four to five orders of magnitude higher than the physiologic concentrations of insulin in the nondiabetic, nonobese human being. The phenomenon is not unique to this particular cell system, with many in vitro systems requiring supraphysiologic concentrations of effector to produce a given response. A number of enzymes capable of metabolizing insulin may be present in this cell system and therefore decrease the effect of insulin. As insulin is also well known to adhere to plastic, the effective concentration may have been decreased by adherence to the culture dishes.

The effect of insulin on the aromatase enzyme system appears to be an increase in maximum velocity. The exact mechanism of this effect remains to be investigated.

Epidermal growth factor was used to investigate specificity because of its growth-promoting effects and known binding to endometrium.[5] The enhancement of aromatase activity by insulin seems to be specific since epidermal growth factor did not have any detectable effect. However, the high concentrations of insulin required to demonstrate an effect suggests that other factors, such as the insulin-like growth factors, may play a role. Studies to investigate such a possible role are currently being made.

These initial findings suggest a number of subsequent studies including steroid-induced insulin receptor dynamics modulating aromatase activity, possible stimulation by other polypeptide hormones, such as gonadotropins and prolactin, and studies throughout the menstrual cycle to determine if a cyclic pattern of response exists.

This study demonstrates that insulin stimulates aromatase activity in both the isolated glands and stroma from human proliferative endometrium. This finding suggests an explanation for the clinical association of

hyperinsulinemia and endometrial neoplasia by an insulin-induced augmentation of endogenous endometrial estrogen production.

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Uterine artery Doppler velocimetry: The significance of divergent systolic/diastolic ratios

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Continuous wave Doppler studies were carried out on both uterine arteries in 71 pregnant women from the twentieth week of gestation onward. Analysis of the waveform included the systolic/diastolic ratio and the presence or absence of a diastolic notch. In the current study, these ratios from 31 women with left/right systolic/diastolic difference, (between left and right uterine arteries) were compared with those of women having normal ratios. A normal left/right systolic/diastolic ratio difference of 0.3 with SD of 0.3 was found. When the left/right difference was plotted against the left/right averaged systolic/diastolic ratio, a correlation coefficient of 0.7 was noted ($p < 0.001$). Significant outcome differences were noted between normal and abnormal left/right difference systolic/diastolic ratios in the perinatal parameters of gestational age at delivery, fetal weight, pregnancy-induced hypertension, proteinuria, and intrauterine growth retardation. Divergent uterine artery ratio findings are a result of one artery being the dominant supplier to the placenta. The majority of women with an elevated systolic/diastolic ratio seem to have divergent uterine blood supply to the uterus and placenta. These data suggest that errors in placentation site contribute to the development of preeclampsia and growth retardation in the fetus. (AM J OBSTET GYNECOL 1987;157:1539-42.)

Key words: Uterine artery, Doppler, ultrasound, systolic/diastolic ratio

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In previous studies we noted that when there was an elevated uterine artery systolic/diastolic ratio as measured by a continuous wave Doppler system, an adverse perinatal outcome[1] and a maternal hypertensive syndrome were likely. From the beginning of the study we measured the waveforms from both the left and the

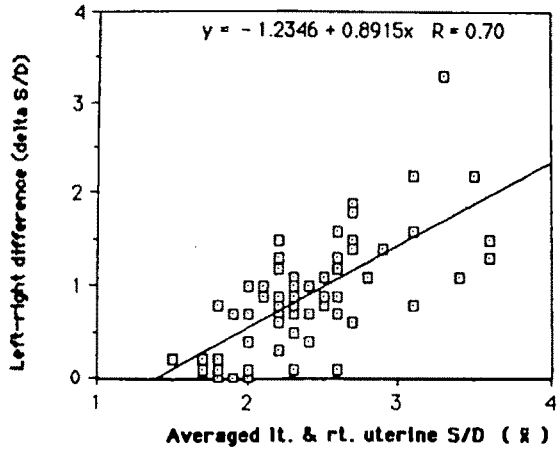


Fig. 1. Data from left and right uterine arteries.

right uteroplacental artery, calculated a systolic/diastolic ratio, and averaged the values from both sides.[2] The reason for making these measurements was because it was noted that in some cases there appeared to be considerable differences between the systolic/diastolic ratio of the left and right vessels. When this occurred, the difference was frequently associated with implantation of the placenta on the lateral wall of the uterus. The vessel on the same side of the placenta demonstrated the lower systolic/diastolic ratio with the disappearance of the diastolic notch.

As our number of observations increased, we began to suspect that in some cases of pregnancy-associated hypertension, the regression to the early pregnancy waveform occurred first in the antiplacental vessels. In this article we will present an analysis of the normal and abnormal divergence of the uterine artery systolic/diastolic ratio and the associated consequences of such an event.

Material and methods

Doppler studies were carried out with a continuous wave transducer with a 6.5 mW/cm^2 power density. The woman was examined in the supine position with a tilt to the left or right, depending on which vessel was being studied. The probe was directed to the paracervical area in the lower quadrants at the site of the uterine vessels. Four waveforms equal in intensity and of maximum size were obtained from each side. A systolic/diastolic ratio was computed and these values were totaled and averaged. Umbilical artery velocimetry was calculated at the same time. All vessels were identified by pattern recognition. Real-time ultrasound was used when we had difficulty in locating the desired vessel pattern. Previously it had been determined that the interobserver error for this examination is 4% with a maximum potential error of 10%. One hundred women with normal pregnancy outcomes were studied prospectively to establish normal values for the left and right uterine arteries and to compute the range of difference between the two. These studies were carried out from the twentieth week onward and were done monthly. Forty subjects from this group were selected randomly and compared with 31 women with elevated systolic/diastolic ratios seen concurrently during the same time period. The women with elevated ratios were examined at least twice with a minimum of a 3-day interval.

Analyses consisted of regression curves, correlation coefficients, and Student's t tests. An Apple computer with a statistical software system was used for these calculations. In this series velocimetry data were not used to influence clinical management.

Results

Fig. 1 depicts the regression curve analysis of the left/right difference in the systolic/diastolic ratio and the averaged systolic/diastolic ratio from both sides. It was determined from our prospective studies that the normal difference, Δ, between the two uterine systolic/diastolic ratios was 0.3 with an SD of 0.3. Hence "abnormal" was defined as ≥ 1 (mean + 2 SD rounded to 1). In previous studies it was determined that the abnormal averaged systolic/diastolic ratio was >2.6 after 26 weeks' gestation. The correlation coefficient was 0.7 between the Δ and averaged values, which was highly significant.

Next, the four combinations of vessel patterns were evaluated with the normal and abnormal Δ and averaged systolic/diastolic values were used as the matrix (Table I). In this way it was hoped to determine which was the more significant variable, the Δ value or the elevated systolic/diastolic ratio.

Table I reveals that only three women with an elevated systolic/diastolic ratio had normal Δ values of <1. Thus, 81% of the women with elevated systolic/diastolic ratios had a significant divergence in flow velocity between the right and left vessels. In Table I those women with normal ratios and Δ values were used as the control population in this study, but the fact that 14% had hypertension and 14% had pregnancy-induced hypertension was noted. Those women with either abnormal Δ values or abnormal ratios experienced less than optimal pregnancy outcomes and perinatal courses when compared with the group with normal ratios and Δ values.

Both groups with abnormal Δ values had earlier deliveries and smaller infants. The umbilical systolic/diastolic ratios in most of these small infants were elevated; this finding is similar to the data reported previously.[3] The combined abnormal group had more instances of pregnancy-induced hypertension. A normal uterine systolic/diastolic ratio but abnormal Δ was

Table I. Outcome in four combinations of uterine artery ratios

	Normal S/D ratio; normal L/R difference (n = 40)	*Abnormal S/D ratio; normal L/R difference (n = 3)*	*Normal S/D ratio; abnormal L/R difference (n = 15)*	*Abnormal S/D ratio; abnormal L/R difference (n = 13)*
Gestational age (wk)	39 ± 1.9	38 ± 2	37.4 ± 3.7	36.6 ± 3.7*
Birth weight (gm)	3364 ± 576	3033 ± 1048	2701 ± 920*	2452 ± 856†
Abnormal nonstress test (%)	22	33	41	10
Fetal distress (%)	5	–	15	27*
Cesarean section (%)	33	67	53	47
Intrauterine growth retardation (%)	3	33	25*	39†
Delivery <37 wk (%)	5	33	33*	47†
Pregnancy-induced hypertension (%)	14	33	33	73†
Proteinuria (%)	9	–	36*	56†
Chronic hypertension (%)	14	–	27	33
Umbilical S/D ratio	2.5 ± 0.4	2.7 ± 0.9	3 ± 1.5	3.2 ± 1.3
Uterine S/D ratio	2.1 ± 0.3	3 ± 0.2	2.4 ± 0.2	3.1 ± 0.4†

S/D = Averaged systolic/diastolic ratio. L/R = left-right S/D ratio difference.
*$p < 0.05$ when compared with normal systolic/diastolic ratio and normal left/right difference, column 1.
†$p < 0.01$ when compared with normal systolic/diastolic ratio and normal left/right difference, column 1.

associated with significantly less pregnancy-induced hypertension ($p < 0.05$) and a trend to less intrauterine growth retardation. Also, fewer deliveries occurred before 37 weeks than occurred when both parameters were abnormal.

We examined the relationship between the lower systolic/diastolic ratio and the ultrasonic placental location and could not demonstrate a clear correlation. The majority of placentas were anterolateral or posterolateral and blood supply to the placentas was variable in these women. If the placenta was exactly lateral, the systolic/diastolic ratio was initially lower on that side. In the 28 women with elevated left/right differences analyzed in terms of placental site, half of the sites were on the left lateral uterine wall and the other half on the right wall.

Comment

Previous reports from our laboratory and others have demonstrated the relationship between abnormal uterine artery velocity waveforms and maternal hypertensive syndromes. Campbell et al.[4] and Trudinger et al.[5, 6] primarily studied the subplacental arcuate vessels identified by real-time ultrasound. Campbell et al.[7] in a recent prospective report examined both vessels but attempted to classify them as placental and antiplacental. We think this is an error because the development of collaterals is unpredictable, and we could not develop a correlation between them because of the large number of anterolateral-posterolateral implantations. When the placenta is anterolateral or posterolateral, there may be also uterine levorotation or dextrorotation, which make the location of the uterine vessels uncertain.

It is interesting that when abnormal differences between the left and right vessels exist, the abnormal differences appeared to have an adverse impact on the pregnancy even when the combined ratio was normal. When both parameters were abnormal the effect on the pregnancy was even more significant. These data, then, would suggest that dissimilar uterine flow velocity may be a significant pathophysiologic event. Noteworthy, also, is the fact that few women had an elevated systolic/diastolic ratio without having divergent velocities between the left and right arteries.

The existence of divergent blood flow velocity may be unique to human beings. Most animals have a two-horned uterus, with only one vessel serving the placenta or placentas. In primates, there is often a bilobed placenta, which probably mandates trophoblastic invasion into the spiral arteries of both uterine vessels. This invasion of the spiral arteries, together with the resultant destruction of the media of the artery, is the probable cause of the lowered uterine artery resistance, the lowering of the systolic/diastolic ratio, and the diastolic notching. One could speculate that the absence of a natural toxemic animal model could be accounted for by this unique vascular relationship.

Medical literature is rich with studies attempting to demonstrate a relationship between the placental blood supply and the development of pregnancy-induced hypertension. Little and Friedman[8] could not demonstrate a relationship between placental location and clinical disease. Chesley[9] has reviewed the experiments carried out in this century on animals and humans in which alteration of uterine blood flow was studied or created to assist in the understanding of pregnancy hypertension. He concluded: "... placental ischemia typically is associated with preeclampsia but it may be secondary rather than causative."

Until Doppler technology was developed it was not possible to study uterine blood flow sequentially

throughout pregnancy. Doppler velocity studies have demonstrated that a predictable pattern of change as pregnancy progresses exists, a pattern completed at the twenty-sixth week of gestation. Pregnancy-induced hypertensive syndromes and fetal growth retardation are seen when these expected changes do not occur or when they do evolve and regress to an earlier pattern.

Some pregnancy hypertension syndromes are seen when primary reduction of velocity flow on the fetal side of the placenta occurs, with normal maternal vasculature.[10, 11] Hypertension not appearing until the pregnancy is near term frequently shows no evidence of diminished maternal or fetal flow velocity.

These data suggest that some women with pregnancy-induced hypertension have a dominant unilateral placental blood flow. Whether this condition and the associated adverse perinatal outcome are amenable to modification will be determined by future studies on placentation and clinical prospective trials.

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The effects of membrane permeability and binding by human serum proteins on steroid influx into the rabbit uterus

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The effects of capillary membrane permeability and binding by human plasma proteins on the influxes of tritium-labeled steroids from the circulation into the uterus were measured in vivo in anesthetized rabbits with a tissue-sampling, single injection technique, and the results were compared with similar studies involving rats. Rabbits were used in this study because this species, unlike the rat, has a sex steroid–binding protein. The impact of capillary membrane permeability markedly varied with different steroids and did not completely parallel the lipid solubility of these compounds. The addition of plasma proteins to the injection vehicle had varied inhibitory effects on the in vivo extractions by the uterus. These in vivo extraction values could not be predicted from in vitro studies of steroid binding to plasma proteins and the results underscore the importance of performing in vivo studies so that we may have a better understanding of the bioavailability of steroids to tissues. (Am J Obstet Gynecol 1987;157:1543-9.)

Key words: Steroids, microvasculature permeability, plasma proteins

We have emphasized in previous studies[1, 2] that factors affecting the differential availability of steroids in the circulation to tissues (e.g., the capillary permeability barriers and plasma protein–binding effects) are important determinants of the biologic responses exerted by these hormones. Our work relates to factors controlling the exit of steroids from the microcirculation to the extravascular space and does not address issues relating to the intracellular environnment. These earlier studies were performed in the rat,[1, 2] a species that lacks a specific sex steroid–binding serum protein.[3] The present study was undertaken in order to evaluate whether the control mechanisms of hormone delivery observed in rats are applicable to another species. In the study, a model was developed to assess transport of circulating steroids into the uterus of the rabbit. This species was chosen because it has an endogenous circulating sex steroid–binding protein (r-SBP),[4] and the uterus was selected since the rabbit spontaneously develops endometrial cancer.[5] Thus, the mechanisms that influence transport of circulating estrogens into this organ may be important in the initiation of this disease.

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Material and methods

The following isotopes with the manufacturer's specific activities were purchased from New England Nuclear, Boston, Mass.: n-[1-^{14}C]butanol, 1.0 mCi/mmol; [2,4,6,7-N-^{3}H]estrone, 88.5 Ci/mmol; [2,4,6,7,16,17-N-^{3}H]estradiol, 140 Ci/mmol; [2,4,6,7-N-^{3}H]estriol, 105 Ci/mmol; [6,7-N-^{3}H]estrone sulfate, 59.0 Ci/mmol; [1,2,6,7-N-^{3}H]progesterone, 101 Ci/mmol; [1,2,6,7-N-^{3}H]corticosterone, 105 Ci/mmol; [1,2,6,7-N-^{3}H]cortisol, 88.3 Ci/mmol; [1,2,6,7,16,17-^{3}H(N)]testosterone, 141 Ci/mmol; [1,2,4,5,6,7,16,17-^{3}H(N)]-dihydrotestosterone, 172 Ci/mmol; and [^{125}I]iodine, 2 mCi/mmol. Unlabeled N-isopropyl *p*-iodoamphetaminne was a gift from Dr. James Lamb, Medi-Physics Inc., Emoryville, Calif. Bovine serum albumin was purchased from Sigma Chemical Co., St. Louis, Mo. Human serum obtained from pregnant women in the third trimester was pooled and frozen.

The radiochemical purity of all isotopes was assessed by thin-layer chromatography with radiochromatogram scanning (Packard Instrument Co., Downers Grove, Ill.) of a small aliquot of the radiolabeled steroids; only a single radioactive peak was found for each steroid. Labeled steroids and conjugates were supplied by the manufacturer in solvent that was evaporated before the isotopes were mixed in buffered Ringer's solution containing 0.1% bovine serum albumin.

Iodination of the IMP and tritiation of bovine serum albumin. Labeling of IMP was achieved by an [^{125}I]iodine exchange reaction as previously described.[6] The isotope had a specific activity of 150 μCi/μmol and was 95% to 98% radiochemically pure, as judged by

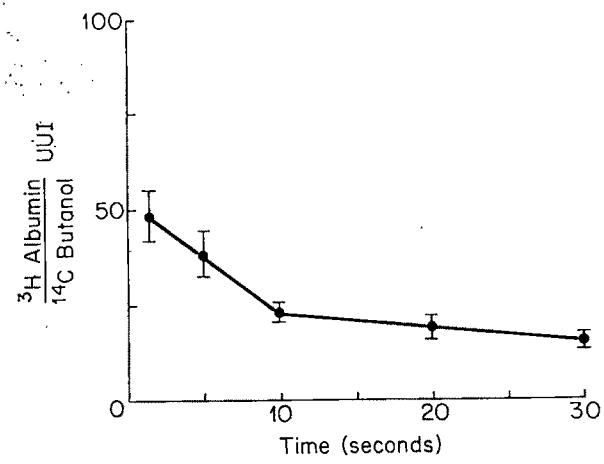


Fig. 1. Mean (±SE) uterus influx index for [^{125}I]bovine serum albumin relative to [^{14}C]butanol at 2, 5, 10, 20, and 30 seconds after injection into the lower abdominal aorta. The values are the mean of four to six animals per point.

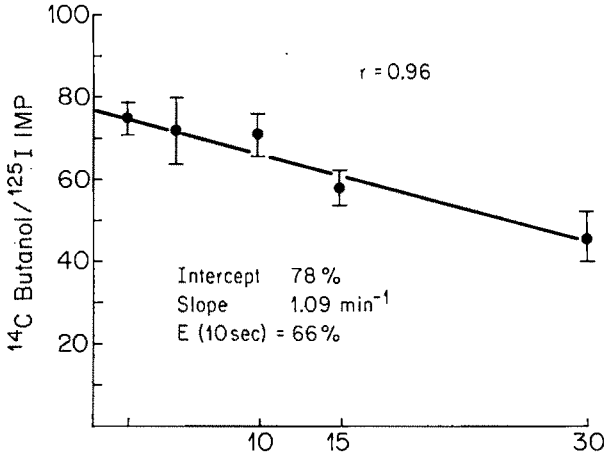


Fig. 2. The ln uterus influx index of [^{14}C]butanol relative to [^{125}I]IMP is plotted versus time after aortic injection. The intercept of 78% indicates that IMP is more diffusible than butanol. The slope of the plot provides the rate constant for butanol washout. The extraction of butanol at 10 seconds is 66%.

thin-layer chromatography.[6] Bovine serum albumin was radiolabeled by reductive methylation and tritiation of albumin lysine residues with [^{3}H]sodium borohydride as previously described.[7] The trichloroacetic acid precipitability of the [^{3}H]albumin was >99%.

The unidirectional influx of test substance into the uterus was measured by a tissue-sampling, single-injection technique similar to that described in studies with rats.[1] The animals used were 4 to 4.5 kg New Zealand White female rabbits. The animals were anesthetized with a mixture of halothane and 100% oxygen, placed in the supine position, and subjected to a vertical laparotomy incision. The right common iliac and left femoral arteries were clamped, and the lower abdominal aorta was isolated. The aorta was cannulated with a 25-gauge needle for rapid (0.5 second) injection of 500 μl of the isotopes in 10 mmol/L Hepes-buffered Ringer's solution (pH = 7.4). The test substances used were either [^{125}I]IMP, [^{3}H]albumin, or the tritium-labeled steroids. The diffusible reference was [^{14}C]butanol.[1, 2] At a predetermined time after the rapid, bolus injection, the uterus was surgically excised. The left uterine horn was minced and about 0.5 to 1 gm of tissue was solubilized in 2 ml Soluene 350 (Packard Instrument Co., Downers Grove, Ill.). An aliquot of the injection solution was similarly prepared for double-isotope liquid scintillation counting. Isotope counts per minute were converted to disintegrations per minute by standard quench corrections. The uterus influx index for [^{3}H]albumin or test [^{3}H]steroid relative to [^{14}C]butanol was calculated as follows:

$$\text{Uterus influx index} = \frac{[^{3}H/^{14}C]\text{uterus}}{[^{3}H/^{14}C]\text{injection solution}} \times 100$$

To ascertain the flow characteristics of the rabbit uterus, the uterus influx index of albumin, which is relatively nondiffusible at the capillary membrane,[8] was determined by injecting 500 μl Ringer's solution containing 5 μCi/ml [^{3}H]albumin and 0.5 μCi/ml [^{14}C]butanol, with tissue sampling at 2, 5, 10, 20, and 30 seconds. Results are presented in Fig. 1. By 10 seconds the uterus influx index of albumin had fallen to a value of 23% and was essentially unchanged at 20 and 30 seconds. This indicated that the injection bolus had been largely cleared from the uterine vasculature by 10 seconds, and whatever albumin that persisted in the uterus at that time represented residual tracer that presumably leaked into the interstitial space or was adherent to the endothelial lining. The washout of butanol from uterine cells from 10 to 30 seconds presumably accounted for the maintenance of the uterus influx index during this period. Therefore, all subsequent studies were performed with tissue sampling 10 seconds after injection, a time sufficiently long to allow substances remaining in the vasculature to leave the organ by way of the venous system but sufficiently short to minimize the loss of compounds passing through the wall of the vasculature into the organ's extravascular space, then reentering the circulation. These compounds are washed out of the organ through the venous system.

The uterus influx index = E_T/E_R where E_T and E_R represent the extractions of the test compound ([^{3}H]albumin, [^{3}H]steroids) and the reference compound ([^{14}C]butanol), respectively, at the appropriate times after injection. The E_T and E_R represent the maximal extraction of unidirectional influx into the uterus,

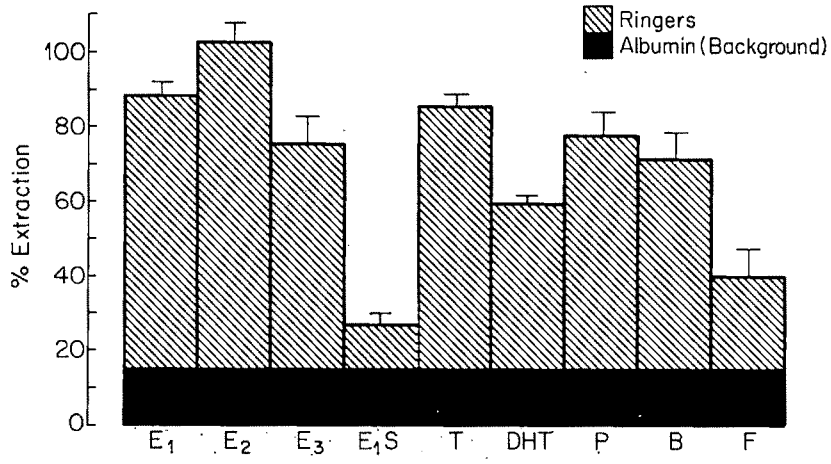


Fig. 3. The uterine extraction (percentage) in the rabbit of labeled estrone (E_1), estradiol (E_2), estriol (E_3), progesterone (P), testosterone (T), dihydrotestosterone (DHT), corticosterone (B), cortisol (F) and estrone sulfate (E_1-S) in Ringer's solution relative to that of [^{14}C]butanol is shown. Data are the mean ± SE (three to eight animals per point). The uterine extraction of albumin refers to nonspecific isotope remaining 10 seconds after injection in the uterine capillaries.

i.e., E (time 0), minus the back diffusion of test or reference compounds during the period between the bolus flow through the uterus and its removal. The uterus influx index may be converted to E_T if E_R is known (E_T = uterus influx index × E_R). It has been assumed that E_R = 1.0 for [^{14}C]butanol.[6] However, recent studies with IMP have shown that butanol, unlike IMP, is not always completely cleared by capillary beds.[6] Therefore, the E_R (time 0) and E_R (10 seconds) for [^{14}C]butanol were estimated in the present studies by comparing the uptake of [^{11}C]butanol relative to [^{125}I]IMP. In preliminary studies, the ln (disintegrations per minute per gram per deciliter injected dose) for [^{125}I]IMP was plotted versus time after injection. No significant difference in values of ^{125}I was found from 2 to 30 seconds, thereby confirming that [^{125}I]IMP, once sequestered by the uterus, does not have a significant efflux back into the microvasculature within 30 seconds, similar to what occurs in the brain.[6] The extraction of [^{14}C]butanol reference was next calculated by determining the uterus influx index of [^{11}C]butanol relative to [^{125}I]IMP at 2, 5, 10, 15, and 30 seconds. A regression line was thus generated (Fig. 2). The intercept at time 0 was the maximal butanol extraction relative to [^{125}I]IMP, i.e., 78%. The extraction of butanol at 10 seconds was found to be 66%. With this value, all uterus influx index determinations were converted to uterine extraction (E_T) values by the formula: E_T = uterus influx index × 0.66. The uterine extraction of albumin at 10 seconds, 15%, was calculated from the uterus influx index for [^{3}H]albumin, 23%, and this value represented the background extraction.

The tracer concentrations of the tritium-labeled steroids varied from 25 to 50 nmol/L, and that of tritium-labeled conjugated hormones ranged from 50 to 100 nmol/L. The ratio of tritium to carbon 14 was kept between 10 and 30. The injection vehicles included buffered Ringer's solution (pH 7.4, 5 mmol/L Hepes) and contained either 0.1% or 4% bovine serum albumin or pooled third-trimester pregnant human serum. The presence of 0.1% bovine serum albumin in the control injections promoted solubility of the labeled steroids in aqueous solutions. When serum was used as the injection vehicle, the concentrations of labeled test substances were deliberately set to be less than the concentration of unbound sex hormone–binding globulin sites, as determined by a selective ammonium sulfate precipitation technique with a [^{3}H]dihydrotestosterone reference.[9]

The fraction of total steroid hormone in human pregnancy serum that was available for influx into the tissues was calculated as follows:

$$\text{Percent available for influx} = \frac{E_{HPS} - E_{BKG}}{E_{control} - E_{BKG}} \times 100$$

where E_{HPS} was the extraction of steroids when human pregnancy serum was used as the vehicle, E_{BKG} was the background value of the method represented by uterine extraction of membrane-impermeable albumin (15%), and $E_{control}$ was the extraction of test substance after injection in Ringer's solution containing 0.1% bovine serum albumin.

The percents of free (dialyzable) fractions of labeled steroids in undiluted human pregnant serum were measured with centrifugal ultrafiltration dialysis.[10] To determine the fraction of steroid that was free and albumin bound, the serum was incubated at 60° C for 30 minutes. The free (dialyzable) fraction was then re-

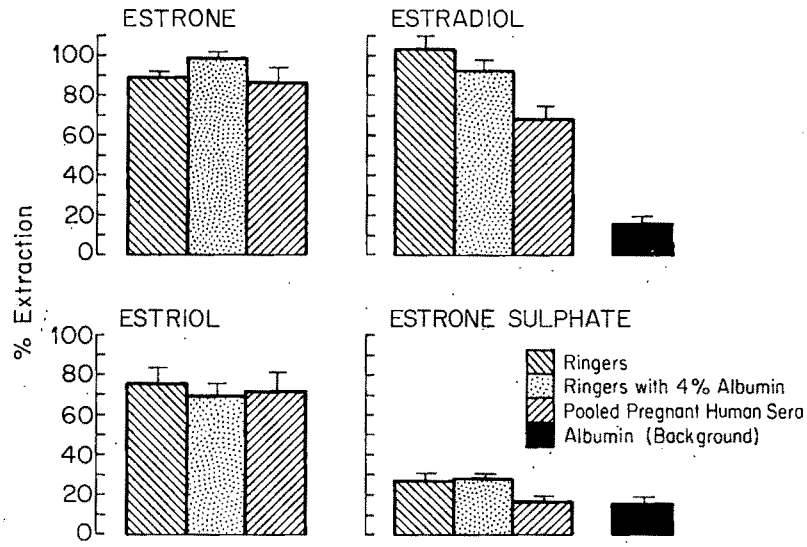


Fig. 4. The mean uterine extractions (percentage) in the rabbit of estrone, estradiol, estriol, and estrone sulfate relative to that of [^{14}C]butanol are shown; injection solutions contained variable amounts of serum proteins.

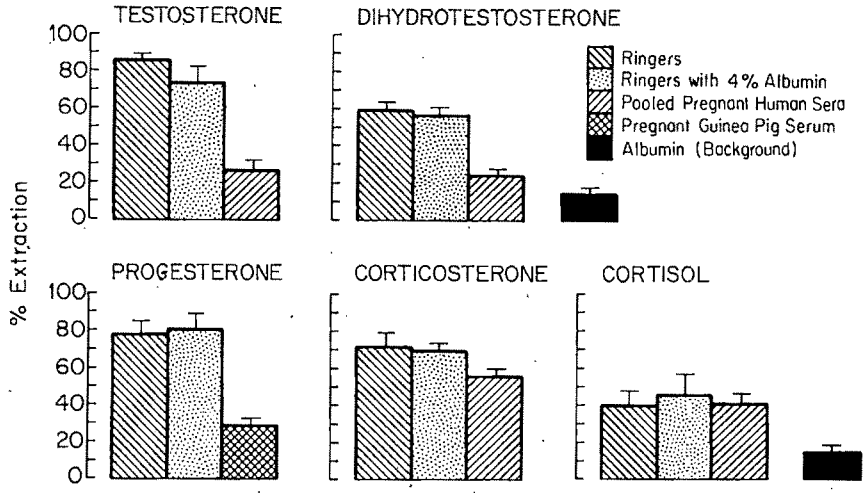


Fig. 5. The mean uterine extractions (percentage) in the rabbit of testosterone, dihydrotestosterone, progesterone, corticosterone, and cortisol relative to that of [^{14}C]butanol are shown; injection solutions contained variable amounts of serum proteins.

determined. The ratio of the dialyzable fraction before incubation to the dialyzable value after heat treatment gave the value of the percent free plus albumin bound.[11]

Statistical analysis of the results was performed with one-way analysis of variance with Duncan's range test or Student's *t* test, where appropriate.

Results

To examine the selective permeability characteristics of the microvasculature of the rabbit uterus, the E_T values of several steroids were determined with Ringer's solution containing 0.1% albumin as the injection vehicle (Fig. 3). Data are mean ± SE (three to six animals per point). The background value of albumin corresponded to the amount shown to remain in the uterine vasculature at 10 seconds. The unidirectional extractions from Ringer's lactate of labeled estrone, estradiol, and testosterone were nearly complete on a single pass through the uterus (i.e., 80%). The extractions of dihydrotestosterone, estriol, progesterone, and corticosterone ranged from 60% to 77%, while those of cortisol and estrone sulfate were reduced at 40% and 27%, respectively.

Fig. 4 shows the mean extractions of different estrogens by the uterus with different injection vehicles.

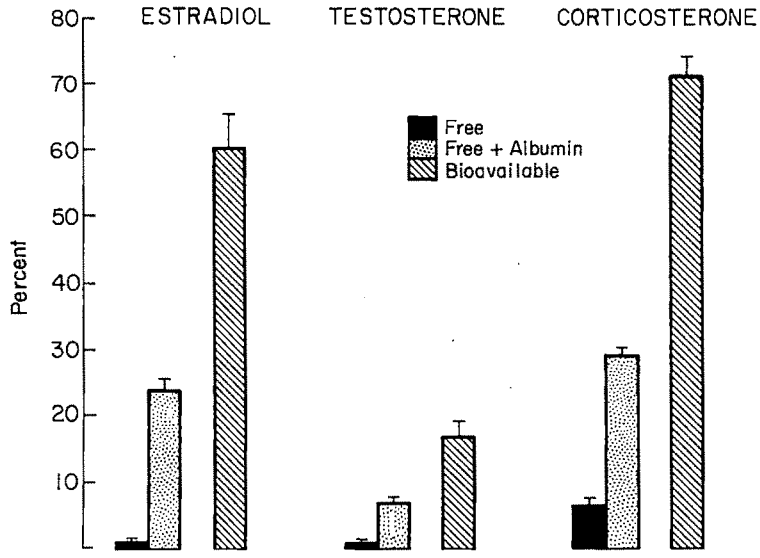


Fig. 6. The percent free (dialyzable) and free plus albumin-bound fractions of labeled estradiol, testosterone, dihydrotestosterone, and corticosterone in undiluted human pregnancy serum in vitro are shown. The fraction of total steroid hormone in human pregnancy serum available for influx into the uterus is also shown.

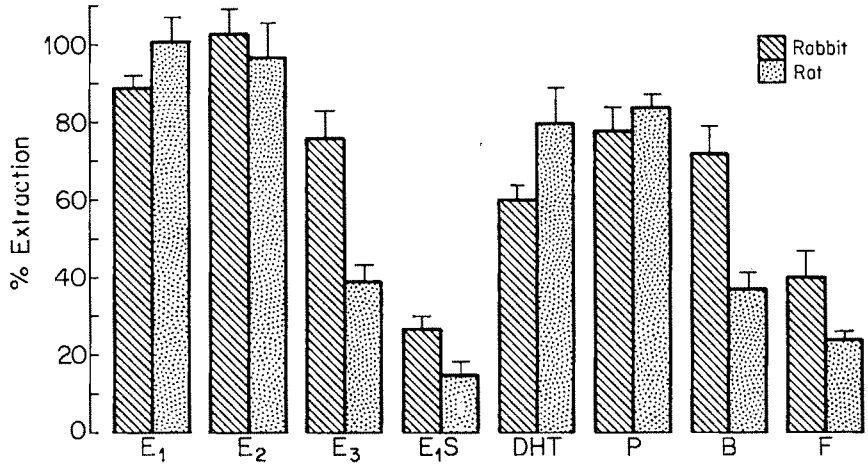


Fig. 7. A comparison between the rabbit (*hatched bars*) and rat (*stippled bars*) uterine extraction (percentage) of labeled estrone (E_1), estradiol (E_2), estriol (E_3), progesterone (P), dihydrotestosterone (DHT), corticosterone (B), cortisol (F), and estrone sulfate (E_1-S) in Ringer's solution relative to that of [^{14}C]butanol is shown. Data are the mean ± SE (three to eight naimals per point). Extraction values include background extraction represented by uptake of [^{3}H]albumin (rabbit) or [^{14}C]dextran (rat).

When albumin was added to Ringner's solution, it did not reduce the influx of estrogens in vivo. When human pregnancy serum served as the injection vehicle, it reduced the influx of estradiol ($p = 0.01$) but did not affect the influxes of estrone or estriol. The extraction of estrone sulfate was reduced to values just above background.

Fig. 5 shows the extractions of progesterone, testosterone, and dihydrotestosterone when different injection vehicles were used. The addition of 4% bovine serum albumin to Ringer's solution had no significant effect on the extractions of these steroids in vivo.[2, 12] With pregnant guinea pig serum as the injection vehicle, the extraction of progesterone was reduced ($p = 0.001$), and with the use of human pregnancy sera, the in vivo uptakes of testosterone ($p = 0.005$) and dihydrotestosterone ($p = 0.001$) were decreased. Fig. 5 also shows the extractions of corticosterone and

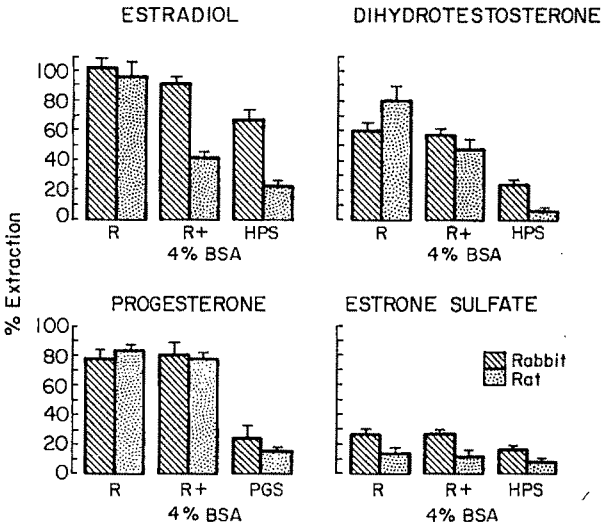


Fig. 8. A comparison between the rabbit (*hatched bars*) and rat (*stippled bars*) of the impact of plasma proteins on the mean uterine extraction of four different classes of steroids, estradiol (C_{18}), dihydrotestosterone (C_{19}), progesterone (C_{21}), and estrone sulfate (steroid conjugate) is shown. *R* = Ringer-Hepes buffer; *BSA* = bovine serum albumin; *HPS* = *human pregnancy serum; PGS* = pregnant guinea pig serum.

cortisol with different injection vehicles. Neither 4% bovine serum albumin nor human pregnancy sera exerted effects on the extractions of these steroids.

The percentages of free (dialyzable) and free plus albumin-bound fractions of labeled estradiol, testosterone, and corticosterone in human pregnancy sera measured in vitro are shown in Fig. 6. The percentages of the same steroids that were bioavailable in vivo are also shown for comparison. The bioavailable fractions of estradiol and corticosterone greatly exceeded the fractions that were free and albumin bound, while the bioavailable testosterone approximated the free plus albumin-bound fractions.

Fig. 7 shows a comparison between the mean uterine extractions of different steroids measured in rabbits and those reported earlier for rats. With the exception of dihydrotestosterone, the percentages of steroids that were bioavailable to the rabbit uterus were the same as or exceeded those injected into the uterus of the rat. However, a parallelism existed between the fractions of each steroid entering the uteri of the two species.

Fig. 8 compares the effects of plasma proteins on the mean extractions of four different classes of steroids, estradiol (C_{18}), dihydrotestosterone (C_{19}), progesterone (C_{21}), and estrone sulfate (steroid conjugate), by the rabbit uterus and the rat uterus, as reported earlier. The impact of plasma proteins on the uptake of these steroids, with the exception of estradiol, was also similar between species. In the presence of bovine serum albumin and human pregnancy serum, much greater fractions of this estrogen entered the uterus of the rabbit.

Comment

In this study, we developed a single bolus injection, double-isotope, timed tissue sampling method suitable for studies in the rabbit model. The use of this technique extended our previous work in rats[1,2] and characterized the role of microvasculature permeability barrier and the effect of plasma protein binding on the unidirectional influx of radiolabeled steroids into the rabbit tuerus. The extraction values measured in this study were *unidirectional* extractions, as opposed to *net* extractions, which are determined from arteriovenous difference measurements. Net extractions mainly reflect cellular steroid metabolism and are a complex function of metabolism, blood flow, cell membrane permeability, and plasma protein binding. Conversely, unidirectional extractions are independent of tissue metabolism of steroids and are therefore more reliable measures of membrane permeability and plasma protein–binding effects.

Marked variability in the influxes of steroids from the circulation into the uterus during a single pass was observed when Ringer's solution was used as the injection vehicle (Fig. 3). These ranged from 103% for estradiol to 27% for estrone sulfate. This differential permeability did not completely parallel the lipid solubility of these compounds as measured by the 1-octanol/Ringer's partition coefficient.[2,12] For example, the partition coefficient for estrone sulfate[2] was threefold greater than for corticosterone,[12] yet the influx of corticosterone was about threefold greater than that of estrone sulfate. The polarity of the compound, which is a function of the hydrogen bond–forming functional groups[13] and the charged functional groups, appeared to predict membrane permeability much better than the 1-octanol/Ringer's partition coefficient.[2,12] These observations were similar to those made previously with a rat uterine model.[1,2]

The addition of plasma proteins to the injection vehicle had varied inhibitory effects on the in vivo extractions of steroids by the rabbit uterus, but these could not be predicted from in vitro studies of steroid binding to plasma proteins. For example, the addition of 4% bovine serum albumin to the injection solution had no inhibitory effects on the in vivo influxes of the steroids tested, although albumin binds to these steroids in vitro.[2,12] Similar discrepancies between the impact of albumin binding of steroids as measured in vivo or in vitro have been reported for the rat uterine model.[1,2]

The presence of sex hormone–binding globulin in the injection vehicle inhibited the influxes of the sex steroids into the rabbit uterus. Greater inhibitions of

testosterone and dihydrotestosterone than of estradiol were observed when human pregnancy serum was the injection vehicle. These findings could be expected from the dissociation constants of these steroids from sex hormone–binding globulin.[14] The presence of progesterone-binding globulin in guinea pig sera also inhibited influx of progesterone into the rabbit uterus.

When the fractions of steroids in human pregnancy sera that were free and albumin bound were estimated in vitro with centrifugal ultrafiltration dialysis[10] and compared with the fractions that were bioavailable in vivo (Fig. 6), it was clear that the bioavailable fractions of estradiol and corticosterone greatly exceeded the fractions that were free and albumin bound. These large discrepancies indicated that estradiol and corticosterone were readily available for influx from the circulating sex hormone–binding globulin and human corticosteroid–binding globulin bound pools, respectively. The bioavailable fraction of circulating testosterone, 17% ± 2%, only modestly exceeded the free plus albumin-bound testosterone fraction, 7% ± 1% (Fig. 6), which indicated that testosterone was not readily available for uterine extraction from the circulating sex hormone–binding globulin bound pool.

In vitro, estrone sulfate exhibits no binding to sex hormone–binding globulin.[15] Thus, the in vivo inhibition of influx of estrone sulfate into the rabbit uterus with human serum was probably due to its binding to other plasma substances. Our observation that estrone sulfate was not available to the rabbit uterus is similar to that of other workers.[16] The influence of human sex hormone–binding globulin on the uptake of testosterone and of pregnant guinea pig sera on the uptake of progesterone into the rabbit uterus was similar to that reported earlier in rats (Fig. 8).

Although many similarities between the findings made in these two species existed, several differences were found. In general, these differences were of a quantitative nature, with the influence of capillary membrane permeability barriers and plasma protein binding on the influx of steroids being less in the rabbit than in the rat model. Of particular interest were the differences observed between species for estradiol[2] (Fig. 8). In the presence of human pregnancy serum, the fraction of this estrogen entering the rat uterus in vivo was approximately 15%,[2] which was less than the free plus albumin-bound fractions measured in vitro, i.e., 24% (Fig. 6). However, the fraction of circulating estradiol entering the rabbit uterus in vivo was 61% with the same injection vehicle. This finding raises the question whether enhanced availability of estradiol to the rabbit uterus contributes to the development of endometrial cancer in this species.

In conclusion, it was observed that the principles of hormone delivery found in rats were applicable to rabbits, as there was general parallelism between the influences of both capillary membrane permeability barriers and plasma protein binding on steroid influxes into the uteri of these two species. This parallelism suggests that the mechanisms observed in rats are not restricted to that species but may have more universal applications. Differences of in vivo uptake of circulating steroids were also observed between the species studied. These differences underscore the importance of future studies in primates to delineate more clearly steroid transport into tissues of humans.

We thank J. Wang and J. Eisenberg for their excellent technical assistance.

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The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation

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The effects of prolonged mild hypoxemia on the fetus have received little attention. We investigated the response to a decrease in fetal arterial Po_2 of 5 mm Hg or less sustained for a period of 24 hours in the chronically instrumented fetal lamb. Hypoxemia was induced by an infusion of nitrogen via the maternal trachea for 24 hours in 13 pregnant sheep at 122 to 139 days' gestation. Two control periods of air infusion via the maternal trachea for 24 hours were alternated with hypoxemia. Fetal hypoxemia was not associated with any change in fetal pH. During the first and second exposures to hypoxemia, there was an increase in fetal plasma lactate of 22.8 ± 22.3 and 15.3 ± 14.6 mg · dl^{-1} (mean ± SD) above preliminary values ($p < 0.05$) compared with the control period. Fetal plasma cortisol also increased by 5.7 ± 6.1 and 9.5 ± 5.4 ng · ml^{-1} (mean ± SD) ($p < 0.05$) compared with the control period. These results suggest that the fetal lamb at 122 to 139 days' gestation is highly sensitive to small decrements in Po_2 of 5 mm Hg or less. (Am J Obstet Gynecol 1987;157:1550-7.)

Key words: Fetal sheep, mild hypoxemia, Po_2, lactate, cortisol, arginine vasopressin

Fetal hypoxemia is a major cause of perinatal death and neonatal morbidity. Many studies have been conducted in pregnant ewes to investigate the effects of short-term hypoxemia for periods of up to several hours. In general, fetal hypoxemia has been induced by lowering maternal inspired oxygen concentration to 9% to 10%, which results in a reduction in fetal Po_2 of about 10 mm Hg. These studies have contributed significantly to our knowledge of the fetal response to severe hypoxemia with respect to the endocrine,[1-4] cardiovascular,[5] and respiratory systems.[6] However, little is known about the response of the fetus to prolonged mild hypoxemia.

Mild to moderate fetal hypoxemia may occur during a chronic reduction in placental blood flow in association with cyanotic heart disease in the mother or during ascent to altitude. We have also demonstrated that throughout the latter part of pregnancy, the fetus is exposed to regular reductions of Po_2 associated with uterine contractures.[7] Both spontaneous and induced myometrial contractures have been found to be associated with a decrease in fetal Po_2 and the incidence of fetal breathing.[8, 9] Similar changes have been demonstrated when fetal Po_2 was reduced by an average of 4.9 mm Hg after brief occlusions of the uterine arteries.[10] For these reasons, we felt that it was important to study the response of the fetus in late gestation to mild to moderate hypoxemia sustained over many hours.

We have developed a model in the pregnant ewe whereby small, precisely regulated degrees of fetal hypoxemia can be produced by an infusion of nitrogen into the maternal trachea.[11] The advantage of this technique is that the mother can feed and drink without interruption and is apparently unaware of low-grade changes in inspired oxygen concentration. We have now used this model to study the effects of mild fetal hypoxemia maintained for 24-hour periods in the fetal lamb.

Material and methods

Thirteen pregnant ewes were prepared for these experiments. Surgery was performed at 116 to 124 days' gestation under halothane general anesthesia. Polyvinyl catheters were placed in the maternal carotid artery, jugular vein, and trachea. The tracheal catheter (3.5

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Table I. Absolute values in fetal arterial blood on the preliminary day and increments from these values on the 4 days of study expressed as Δ mean ± SD

Value	*Day of experiment*				
	Preliminary	*Δ day 1 air*	*Δ day 2 nitrogen*	*Δ day 3 air*	*Δ day 4 nitrogen*
P_{O_2} mm Hg (n = 16)	23.4 ± 3.58	0.0 ± 1.61	0.0 ± 2.27*	−0.1 ± 2.07	−4.8 ± 2.84*
O_2 saturation % (n = 16)	56.9 ± 11.55	−1.1 ± 3.23	−16.2 ± 6.21*	−2.2 ± 5.70	−17.4 ± 8.90*
Hb gm · dl^{-1} (n = 16)	9.9 ± 0.93	0.1 ± 0.45	0.0 ± 0.63	−0.1 ± 0.66	0.1 ± 0.94
P_{CO_2} mm Hg (n = 16)	49.4 ± 4.14	0.7 ± 1.78	−3.4 ± 3.20*	0.3 ± 3.54	−2.8 ± 4.94*
pH (n = 16)	7.37 ± 0.027	0.01 ± 0.016	−0.01 ± 0.045	−0.01 ± 0.021	−0.03 ± 0.069
Base excess mEq · L^{-1} (n = 16)	2.2 ± 3.23	−0.1 ± 1.57	−2.4 ± 3.73*	−0.7 ± 2.29	−3.0 ± 4.73*
HCO_3^- mEq · L^{-1} (n = 16)	27.3 ± 3.32	−0.4 ± 1.33	−1.5 ± 2.66*	−0.1 ± 3.50	−1.9 ± 3.82
Plasma lactate mg · dl^{-1} (n = 9)	14.3 ± 2.74	1.0 ± 2.16	22.8 ± 22.28*	2.5 ± 2.68*	15.3 ± 14.65*
Plasma cortisol ng · ml^{-1} (n = 8)	3.7 ± 5.62	0.9 ± 5.04	5.7 ± 6.1*	1.6 ± 2.6	9.5 ± 5.4*
Plasma glucose mg · dl^{-1} (n = 9)	21.1 ± 4.70	−0.4 ± 1.43	−1.2 ± 5.57	−0.3 ± 0.66	0.1 ± 0.94
Plasma AVP pg · ml^{-1} (n = 5)	0.7 ± 0.16	0.0 ± 0.19	0.0 ± 0.27	0.3 ± 0.38	0.1 ± 0.28

Hb = Hemoglobin; AVP = arginine vasopressin.
*Indicates significance at $p < 0.05$ with the paired t test.

Table II. Absolute values in maternal arterial blood on the preliminary day and increments from these values on the 4 days of study expressed as Δ mean ± SD

Value	*Day of experiment*				
	Preliminary	*Δ day 1 air*	*Δ day 2 nitrogen*	*Δ day 3 air*	*Δ day 4 nitrogen*
P_{O_2} mm Hg (n = 16)	101.3 ± 6.75	0.8 ± 5.33	−30.4 ± 7.72*	−0.7 ± 7.10	−33.3 ± 6.62*
O_2 saturation % (n = 16)	93.8 ± 1.64	−0.1 ± 0.86	−8.4 ± 3.33*	−0.3 ± 1.77	−11.0 ± 5.70*
Hb gm · dl^{-1} (n = 16)	9.4 ± 1.12	0.0 ± 0.33	0.2 ± 0.78	−0.2 ± 0.86	0.2 ± 0.91
P_{CO_2} mm Hg (n = 16)	34.8 ± 3.43	0.3 ± 2.04	−3.6 ± 2.54*	−0.02 ± 3.41	−2.7 ± 3.85*
pH (n = 16)	7.47 ± 0.037	0.01 ± 0.018	0.02 ± 0.042	−0.01 ± 0.049	0.0 ± 0.044
Base excess mEq · L^{-1} (n = 16)	1.5 ± 3.33	0.0 ± 1.92	−0.4 ± 3.00	−0.5 ± 3.96	−1.4 ± 3.95
HCO_3^- mEq · L^{-1} (n = 16)	24.9 ± 3.03	0.1 ± 1.53	−2.3 ± 3.56*	−0.5 ± 2.35	−2.8 ± 4.43*
Plasma lactate mg · dl^{-1} (n = 10)	4.5 ± 1.20	0.3 ± 1.51	1.7 ± 2.50	1.0 ± 2.04	2.2 ± 2.79*
Plasma cortisol ng · ml^{-1} (n = 8)	11.4 ± 15.73	−1.6 ± 8.91	−1.7 ± 7.96	−1.9 ± 13.57	2.5 ± 10.80
Plasma glucose mg · dl^{-1} (n = 10)	46.1 ± 8.53	0.3 ± 2.64	1.7 ± 5.54	0.2 ± 7.46	2.5 ± 11.79
Plasma AVP pg · ml^{-1} (n = 5)	1.6 ± 0.58	0.1 ± 0.57	0.3 ± 0.76	0.6 ± 0.34*	0.3 ± 0.29

Hb = Hemoglobin; AVP = arginine vasopressin.
*Indicates significance at $p < 0.05$ with the paired t test.

mm outer diameter and 2.5 mm inner diameter) was introduced in the direction of the lungs to a distance of 15 cm into the trachea from a point approximately 2 to 3 cm below the cricoid.[11]

Polyvinyl catheters were introduced into the fetal carotid artery, jugular vein, and amniotic cavity as previously described.[12] Electrodes of stainless steel wire (Cooner wire AS632) were also placed into the cortex of the fetal brain, the eyelids, nuchal muscle, alae nasi, or diaphragmatic muscle and subcutaneous tissue of the thorax to record fetal biophysical variables.[9] In addition, electrodes were also secured to the myometrium to record uterine activity.[9] Catheters and electrodes were exteriorized through the flank of the ewe.

Oxygen tension was measured continuously in fetal carotid arterial blood by means of a miniaturized Clark-type electrode mounted on the tip of a double-lumen catheter (venous oxygen probe, Orange Medical Instrumentation, Ltd., High Wycombe, England)[7] and in subcutaneous tissue by means of custom-made galvanic oxygen electrodes.[13] The results of continuous oxygen measurements, cardiovascular responses, and other biophysical variables will be presented in forthcoming papers.

The experimental protocol began on the fifth to seventh day after surgery and continued for a period of 4 days. On the preliminary day before the experiment, no gas was infused into the maternal trachea. On the first experimental day (day 1), compressed air was infused via the tracheal catheter at a rate of 4 or 6 L/min for a 24-hour period. On the following day, the infusion was switched to nitrogen at 4 or 6 L/min to lower maternal alveolar P_{O_2}. The air and nitrogen infusions were repeated on the third and fourth experimental days, respectively.

Arterial blood samples were obtained from both mother and fetus on the preliminary day before the experimental protocol was begun and thereafter at

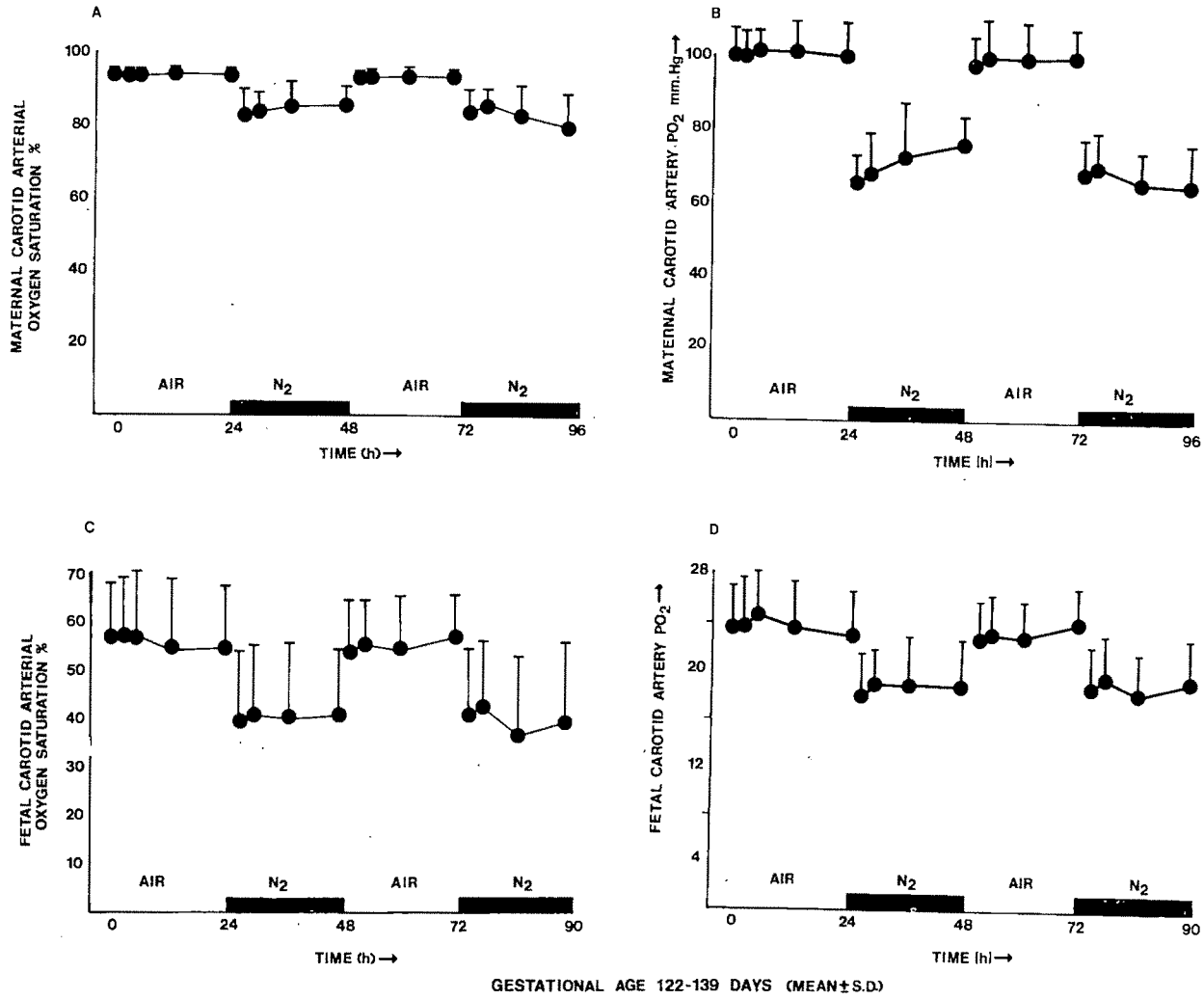


Fig. 1. Maternal arterial oxygen saturation (**A**) and oxygen tension (**B**) and fetal arterial oxygen saturation (**C**) and oxygen tension (**D**) in 16 experiments at 122 to 139 days' gestation (mean ± SD). Nitrogen was infused at 4 or 6 L · min^{-1} via a tracheal catheter in the ewe to produce hypoxemia (■). On control days, air was infused at 4 or 6 ml · min^{-1}.

2, 4, 12, and 23 hours on each day of air or nitrogen infusion. Heparinized blood samples (1 ml) were obtained for measurement of pH, blood gases, oxygen saturation, hemoglobin, and whole blood glucose. Additional samples (1.5 ml) were obtained for measurement of plasma lactate, cortisol, and arginine vasopressin. Fetal red cells were returned to the fetus after centrifugation of the sample and separation of plasma.

The pH and blood gases were measured by means of a Radiometer BMS3 maintained at a temperature of 39° C. Oxygen saturation and hemoglobin were measured with a Radiometer OSM2 hemoximeter. Whole blood glucose and plasma lactate were measured by specific enzymatic techniques with a Yellow Springs Instrumentation analyzer (Model no. 27). Cortisol was measured with a specific radioimmunoassay after methylene dichloride extraction; this method has been validated for measurement of cortisol in fetal and maternal plasma.[14]

Arginine vasopressin in fetal and maternal plasma was measured with a radioimmunoassay after acetone-petroleum ether extraction of plasma. Cold acetone (2 ml) was added to 0.6 ml of plasma, mixed on a multiple tube vortex for 1 minute, and centrifuged at 4000 rpm at 4° C for 20 minutes. The supernatant was decanted into a tube containing 2 ml of petroleum ether, mixed, and allowed to stand for 10 minutes before freezing the aqueous (lower) phase at −60° C in methyl alcohol. The organic phase was poured off, and the aqueous phase, which contains arginine vasopressin, was dried overnight by lyophilization. The extract was resuspended in 1 ml assay buffer (0.02 mol/L of 5,5-diethylbarbituric acid, 0.139 mol/L of sodium chloride, 0.011 mol/L of sodium ethylenediaminetetraacetic

acid, 0.066 mmol/L of L-cystine, and 0.25% of bovine serum albumin (pH 7.4), and 0.45 ml of the reconstituted extract was measured in duplicate in the assay.

Standard curves were prepared by mixing unlabeled arginine vasopressin (Bachem, Torrance, Calif.), which ranged from 0.06 to 8.0 pg in 0.45 ml buffer per tube, and 50 μl of antibody (the Arnel Products Co., New York, N.Y.) to a final dilution of 1:125,000 in a volume of 0.55 ml. After 24-hour incubation at 4° C, 2000 cpm of iodine 125–arginine vasopressin (NEX-128; 2200 Ci/mmol, New England Nuclear, Boston, Mass.) in 50 μl was added, which was followed by further incubation for 48 hours at 4° C. Separation of free iodine 125–arginine vasopressin was carried out after the addition of 200 μl, 2.0% charcoal, 0.75% dextran in 0.1 mol/L of phosphate buffer, pH 7.4, and centrifugation at 4000 rpm. Precipitates containing unbound arginine vasopressin were counted in a Micromedics γ-counter for 5 minutes. Extraction efficiency (70%) was evaluated by extraction of 2 and 4 pg of cold arginine vasopressin from low pool plasma. Maternal and fetal plasma extracts diluted in parallel to the standard curve. Antisera cross-reactivity was <0.01% with oxytocin, 15% with lysine vasopressin, and 31% with arginine vasotocin. Sensitivity of the assay was 0.1 pg per tube. Intra-assay coefficient of variation was 9.4% and interassay coefficient of variation was 12.1%.

Statistical analysis. Gestation at the start of the experiment ranged from 122 to 136 days. Sixteen experiments were performed in 13 ewes; three ewes were subjected to a second experiment 7 to 14 days later. The infusion rate of air or nitrogen via the maternal trachea was set at 4 L/min in nine experiments and 6 L/min in the other seven experiments. All plasma samples were not analyzed for all hormones and metabolites. The number of samples assayed for each variable depended on availability of plasma. For the purpose of statistical analysis, all results available from the 16 experiments have been pooled since there were no significant differences between infusion rates of 4 L of nitrogen and 6 L nitrogen per minute.

Increments from preliminary values on the different days of air or nitrogen infusion were calculated with the use of mean values for the four samples obtained on each day. Initial one-way analysis of variance was performed on the data, followed by the paired t test with Bonferroni correction on the increments from the preliminary values on the different days. The mean ± SD for the increment above the preliminary day on each day was calculated. The paired t test was used to test whether the increment on each day of nitrogen administration was significantly different from zero. Thus two comparisons were made (i.e., the increment on days 2 and 4 were significant). Significance was assumed at $p < 0.05$ (equivalent to 0.025 after Bonferroni correction). To analyze the speed of any changes, the last sample obtained while the trachea was infused with compressed air was compared with the first sample (after 2 hours) of nitrogen infusion on both days 2 and 4 with use of the paired t test. Significance was assessed at the 0.05 level. Unless otherwise stated, data are presented throughout as mean ± SD.

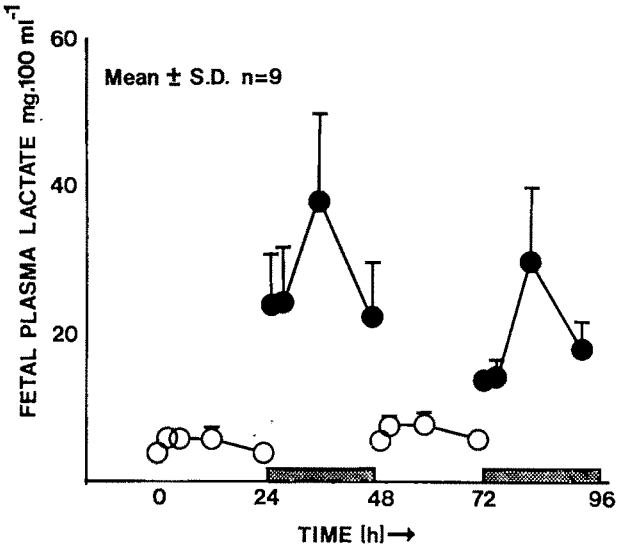


Fig. 2. Fetal plasma lactate in the fetuses of nine ewes at 122 to 132 days' gestation. Air (○) or nitrogen (●) was infused into the maternal trachea at 4 or 6 L · min^{-1}. *Bars* (▬) indicate 24-hour periods of hypoxemia.

Results

Outcome of experiments. One fetus died on the fourth day during nitrogen infusion. The mother had shown increasing uterine activity during the experiment and was in labor at the time of fetal death. All other fetuses survived the experimental period. In one experiment, the ewe showed signs of increasing respiratory distress during nitrogen infusion, which was therefore discontinued approximately 12 hours before the end of the fourth day's protocol. In one other experiment, the nitrogen infusion was accidently disconnected during the last 12 hours of infusion on day 4. The results of blood samples obtained during these periods have therefore been excluded from the final analysis.

Changes in fetal and maternal pH, blood gases, and hemoglobin. The absolute values for the fetal and maternal variables averaged for the preliminary day and the increments for each day of the experimental period are shown in Tables I and II. Both maternal and fetal arterial P_{O_2} and oxygen saturation decreased significantly on days 2 and 4 during nitrogen infusion. There was no significant change in maternal and fetal pH or hemoglobin. Maternal and fetal P_{CO_2} decreased on

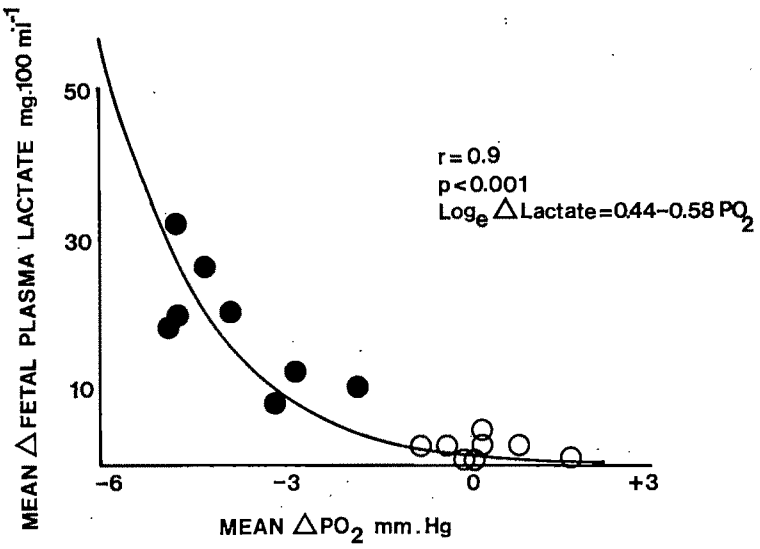


Fig. 3. Relationship between change in mean plasma lactate and change in mean PO_2 in fetal carotid arterial blood compared with preliminary day when no gas was infused. *Open circles* (○) indicate samples obtained during infusion of air, and *closed circles* (●) indicate samples obtained during infusion of nitrogen via a maternal tracheal catheter. Mean values for all fetuses at each of the 16 data points on days 1 to 4 are shown.

days 2 and 4 during nitrogen infusion. Hypocarbia was almost certainly caused by maternal hyperventilation as a result of hypoxemia.

Fig. 1 illustrates the changes in PO_2 and oxygen saturation (mean ± SD) in maternal and fetal blood for all 17 samples obtained during the 5-day experimental protocol. It can be seen that the decrease in maternal arterial PO_2 of about 30 to 35 mm Hg was accompanied by only a small decrease in oxygen saturation of <10%. On the other hand, in the fetus, the decrease in PO_2 was only about 5 mm Hg, but was accompanied by a larger decrease in oxygen saturation than in the mother, that is, about 15%.

Fetal and maternal blood glucose and plasma lactate. Fetal and maternal blood glucose values did not vary significantly on any of the experimental days. However, fetal plasma lactate was significantly elevated on days 2 and 4 during nitrogen infusion. Maternal plasma lactate values showed little change except for day 4 when the small increase in lactate achieved statistical significance (Table II). The fetal lactate values for each of the 17 samples obtained during the 4-day protocol are shown in Fig. 2. It can be seen that fetal plasma lactate values had increased more than twofold within 2 hours ($p < 0.05$) of the start of nitrogen infusion, peaking at greater than 12 hours and tending to decrease toward normal at greater than 23 hours.

The relationship of mean ΔPO_2 to mean Δ lactate values in the fetus for all 16 samples during the experimental period is shown in Fig. 3. Fetal lactate values increase significantly as a function of decreasing PO_2. The exponential best fit equation shows that fetal plasma lactate rose 2.8 $mg \cdot dl^{-1}$ when the PO_2 fell 2 mm Hg. The rise in fetal plasma lactate was 21 $mg \cdot dl^{-1}$, for a 5 mm Hg fall in PO_2.

Fetal and maternal plasma cortisol. Concentrations of fetal plasma cortisol increased significantly on days 2 and 4 during nitrogen infusion ($p < 0.05$), but no significant changes occurred in maternal cortisol values (Tables I and II). Plasma cortisol values for both mother and fetus (mean ± SEM) in all 17 samples obtained during the 4-day protocol are shown in Fig. 4. It can be seen that there was wide variation in both vascular compartments, particularly in the mother. However, in the fetus, the increase in plasma cortisol was more consistently related to the onset of hypoxemia and was statistically significantly elevated by 2 hours on days 2 and 4 ($p < 0.05$).

Fetal and maternal arginine vasopressin. No changes were observed in either maternal or fetal arginine vasopressin values during the 4 day protocol (Tables I and II).

Comment

Most studies on the effect of hypoxemia on the fetus have been relatively short-term, that is, in the order of hours rather than days. Furthermore, in most studies fairly severe maternal and fetal hypoxemia has been induced by reducing maternal inspired oxygen concentration to about 50% of normal. Under these con-

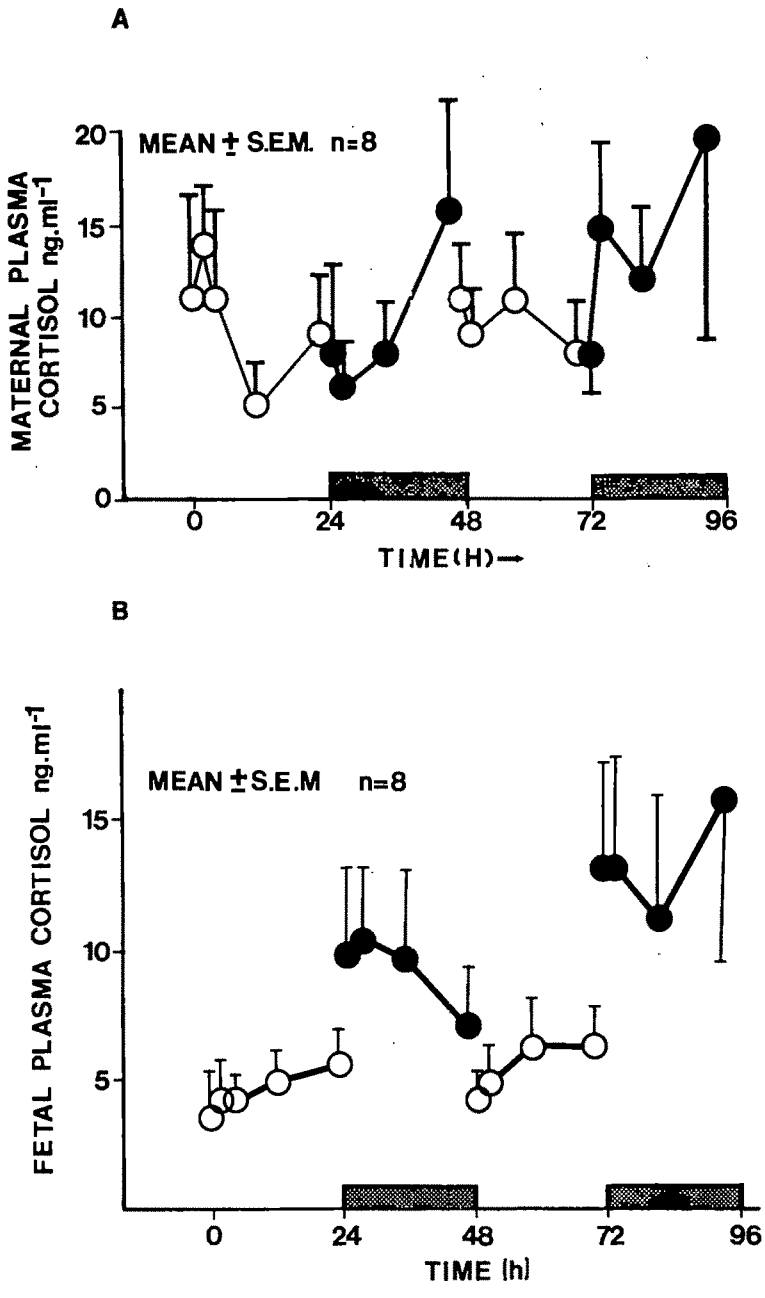


Fig. 4. Maternal arterial plasma cortisol concentration (**A**) and fetal carotid arterial plasma cortisol concentration (**B**) in eight experiments at 122 to 139 days' gestation. *Open circles* (○) indicate samples obtained during infusion of air and *closed circles* (●) during infusion of nitrogen to produce hypoxemia (▬).

ditions, fetal P_{O_2} falls by 50%, and fetal acidosis develops to varying degrees, depending on the duration of hypoxemia.

Our experiments were directed toward obtaining an understanding of how well the fetus tolerates prolonged mild hypoxemia. We considered this to be important from the following points of view: (1) The fetus undergoes multiple episodes of mild hypoxemia with drops in arterial P_{O_2} of 2 to 8 mm Hg at regular intervals during late gestation in association with uterine contractures; (2) the ascent to altitude during pregnancy is associated with mild maternal-fetal hypoxemia depending on the level of ascent, and (3) persistent mild-to-moderate hypoxemia is believed to occur in association with intrauterine growth failure in the clinical situation.

Our data show that prolonged mild fetal hypoxemia resulting from maternal hypoxemia was well tolerated by the fetus but leads to significant metabolic responses by the fetus or the placenta that were not observed in the mother. Fetal plasma lactate increased significantly. It appears that hypoxemia induced by lowering inspired P_{O_2} in the mother is tolerated better by the mother than by the fetus. This is not altogether surprising in view of the difference in maternal and fetal oxygen dissociation curves. A decline in P_{O_2} of 35 mm Hg in the mother led to a decrease in oxygen saturation of <10%, whereas a decline of only 5 mm Hg in the fetus led to a decrease in oxygen saturation of 15% or more. This represents a percentage change of nearly 30% in the fetus compared with only 10% in the mother. Oxygen availability to tissues depends on the amount of oxygen carried by hemoglobin and the P_{O_2} gradient from plasma to tissues. It is apparent that the reduction in fetal oxygen availability in our experiments was sufficient to cause tissue oxygen deprivation and metabolic effects in the fetus. We do not have any data to show where the lactate that appeared in fetal plasma was being produced, but it is possible that a decrease in blood flow to fetal muscles and other organs in response to mild hypoxemia was sufficient to trigger anaerobic metabolism and lactate production. The possibility that placental lactate production is altered by small changes in P_{O_2} or oxygen content is an intriguing one that should be investigated.

There was no significant change in fetal pH during hypoxemic periods despite a significant change in fetal lactate. This was probably accounted for by a reduction in P_{O_2} in both maternal and fetal compartments caused by maternal hyperventilation.

Significant changes were observed in cortisol concentrations of both maternal and fetal plasma during the hypoxemic episodes. This increase was significant in fetal plasma within 2 hours of the start of the hypoxemia. However, perusal of the data presented in Fig. 4 shows that there was wide variation in the cortisol response, particularly in the maternal compartment. Cortisol has been shown to cross the placenta in both directions, and it is possible that in certain situations, fetal values could be a reflection of those in the mother. However, placental transfer does not usually occur unless maternal cortisol values exceed 44 ng · ml^{-1}.[15] Maternal cortisol in our ewes rarely exceeded these values, and fetal values increased within 2 hours of the first exposure to hypoxemia at a time when maternal values were not increased (Fig. 4).

The plasma cortisol values found in both mother and fetus during hypoxemic episodes were considerably lower than those reported by other investigators during hypoxic episodes in the ewe. Boddy et al.[1] exposed pregnant ewes to 9% oxygen. Their increase in maternal plasma cortisol ranged from 18.7 to 145 ng · ml^{-1} and from 10.7 to 30.4 ng · ml^{-1} in fetal plasma cortisol. They also observed that maternal adrenocorticotropic hormone increased about 2.5-fold during hypoxemia, whereas fetal adrenocorticotropic hormone increased about 14-fold. The effect of progressive fetal hypoxemia and acidosis induced by administration of a hypoxic gas mixture (9% oxygen) to the pregnant ewe over a period of 7.2 hours was also studied by Challis et al.[4] In contrast to Boddy et al.,[1] they found little change in maternal adrenocorticotropic hormone or cortisol levels, but a large increase in fetal adrenocorticotropic hormone and cortisol values occurred within 3 hours of sustained hypoxemia.

Our observations suggest that the fetal pituitary adrenal axis at 122 to 139 days' gestation is quite sensitive and responds to mild hypoxemia (ΔP_{O_2} of -5 mm Hg) by releasing cortisol. We cannot entirely exclude some transplacental contribution from the maternal compartment, but as previously mentioned, this is unlikely. Other investigators[16] have shown that the direction of transfer of cortisol across the placenta is more likely to be from fetus to mother than in the reverse direction. The modest increase in fetal cortisol values, that is, about twofold, in our experiments is higher than that observed with moderate fetal hypoxemia induced by a reduction in uterine blood flow for 2 hours.[3] In the latter experiments, mean fetal cortisol values increased by 1.3 times for all gestational ages combined, but the largest increase (>200%) was observed in fetuses of 120 to 138 days. All but one of our fetuses fell into this gestational age range, which may help explain the apparent sensitivity of the fetal adrenal gland observed in our experiments. Finally, arginine vasopressin values were not increased in either mother or fetus, suggesting that the posterior pituitary gland is not sensitive to mild reductions in fetal P_{O_2} of this magnitude.

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Fetal heart rate response to vibratory acoustic stimulation predicts fetal pH in labor

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Vibratory acoustic stimulation was performed during labor in 188 instances 60 seconds before fetal scalp puncture was done to determine fetal scalp blood pH. The fetal heart rate response was recorded for both vibratory acoustic stimulation and fetal scalp puncture. No instance of fetal acidosis occurred in the presence of an acceleration to either vibratory acoustic stimulation or fetal scalp puncture. Vibratory acoustic stimulation was more likely to elicit an acceleration than fetal scalp puncture in the nonacidotic fetus. Vibratory acoustic stimulation is less invasive and may be used in some instances in which fetal scalp blood puncture for pH determination is technically impossible. (AM J OBSTET GYNECOL 1987;157:1557-60.)

Key words: Vibratory acoustic stimulation, fetal pH, fetal heart rate monitoring

Accelerations in fetal heart rate induced by vibratory acoustic stimulation of the fetus have been associated with fetal well-being during both the antepartum and intrapartum periods.[1-4] The increase in heart rate observed is thought to be related to a change in the state of arousal, which is manifested in a startle response of the fetus similar to that observed in the neonate.[5,6] Loss of the startle reflex in the neonate may indicate altered neurologic function or acidosis. Direct fetal scalp stimulation that results in an acceleration in heart rate has also been correlated with a normal acid-base status.[7] This study was undertaken to examine the relationship between vibratory acoustic stimulation, direct fetal scalp stimulation, and fetal scalp blood pH.

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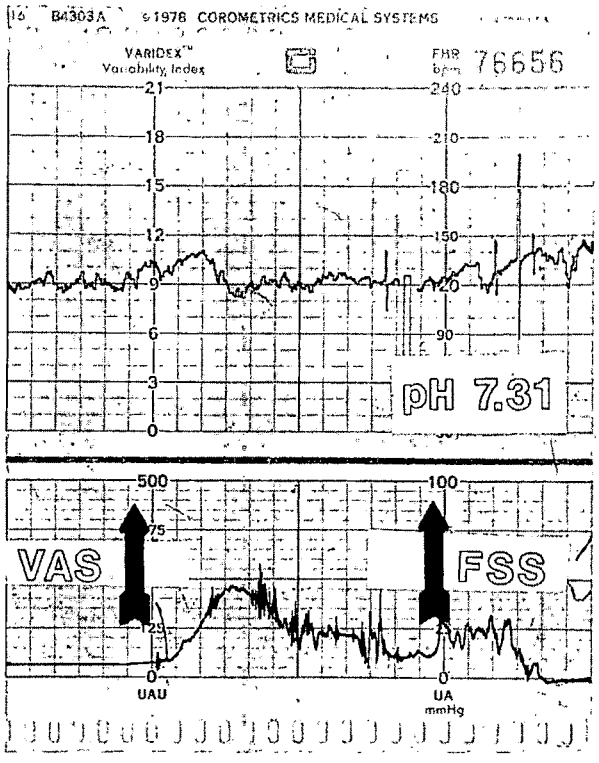


Fig. 1. Fetal heart rate accelerations occurring in response to both vibratory acoustic stimulation *(VAS)* and fetal scalp puncture *(FSS)* with a fetal pH of 7.31.

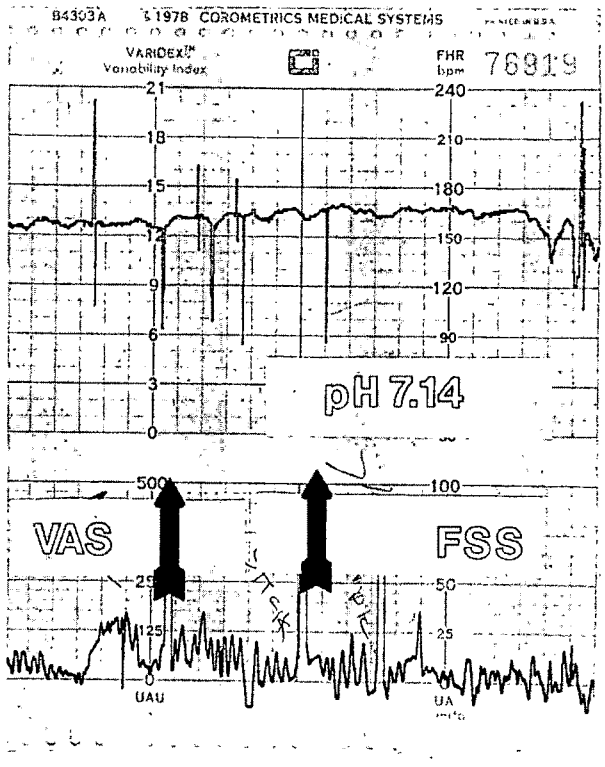


Fig. 2. Lack of fetal heart rate acceleration with either stimulus with a fetal pH of 7.14.

Table I. Indications for fetal pH determination

Indication	*No. of samples*
Late decelerations	96
Severe variable decelerations	52
Bradycardia	34
Tachycardia	25
Decreased variability	13
Meconium	5
Sinusoidal pattern	2
Saltatory pattern	2

Material and methods

This study was undertaken at The New York Hospital–Cornell University Medical College from March 1985 to March 1986. All patients included were in active labor with ruptured membranes and had evidence of abnormal fetal heart rate tracings. Liberal guidelines for performing fetal pH determinations are used on our service; those patients with suspicious or equivocal tracings have a pH measurement obtained. In addition, any patient with meconium plus a fetal heart rate abnormality such as decreased beat-to-beat variability or fetal tachycardia had a fetal scalp blood pH measurement performed. All patients were 34 weeks' gestation or greater. The fetal heart rate was monitored continuously by means of the Corometrics 112 fetal heart rate monitor. Sixty seconds before determination of scalp blood pH, a single 3-second vibratory acoustic stimulus was applied over the fetal vertex with the use of the Western Electric Model 5c electronic artificial larnyx. Fetal heart rate was observed for 60 seconds and fetal scalp blood was then obtained by the standard puncture technique and analyzed on a Corometrics 220 pH system. Fetal heart rate response to both vibratory acoustic stimulation and fetal scalp stimulation was recorded and correlated with the pH value obtained. An acceleration was defined as an increase in fetal heart rate above the baseline of 15 bpm sustained for 15 seconds occurring within 60 seconds after either stimulation.

Results

Fetal heart rate response to both vibratory acoustic stimulation and fetal scalp stimulation was recorded in 183 instances in 127 patients. Typical fetal heart rate recordings in both nonacidotic and acidotic fetuses are shown in Figs. 1 and 2. The indications for fetal pH determination are listed in Table I, with more than one indication present in some patients. Fetal bradycardia was defined as a decrease in fetal heart rate of >30 bpm lasting for more than 60 seconds that had resolved before the pH determination. Table II lists the accel-

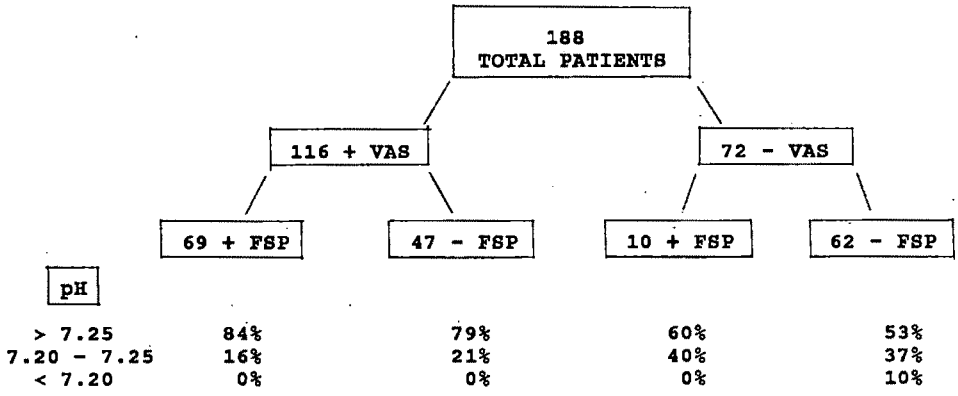


Fig. 3. The incidence of acceleration (+) and no acceleration (−) to vibratory acoustic stimulation *(VAS)* and subsequent fetal scalp puncture *(FSP)* divided by fetal scalp blood pH.

Table II. Response of fetal heart rate to fetal stimulation according to fetal pH

	Vibratory acoustic stimulation		*Fetal scalp puncture*	
Fetal pH	*Acceleration (%)*	*No acceleration (%)*	*Acceleration (%)*	*No acceleration (%)*
>7.25	95 (70.9)	39 (29.1)	64 (47.8)	70 (52.2)*
7.20-7.25	21 (43.7)	27 (56.3)	15 (31.2)	33 (68.8)
<7.20	0	6 (100)	0	6 (100)

*$p < 0.001$.

eration response to each stimulus according to the pH value obtained from the fetal blood sample. Fig. 3 shows the percentage of patients responding to each stimulus and the pH values of each group. No instances of fetal acidosis (pH < 7.20) occurred in the presence of an acceleration in fetal heart rate to either vibratory acoustic stimulation or fetal scalp stimulation. Vibratory acoustic stimulation was more likely to elicit an acceleration than was fetal scalp stimulation in the nonacidotic fetus (pH > 7.25).

A fetal heart rate acceleration to vibratory acoustic stimulation has a sensitivity of 63.7% and a specificity of 100% in identifying the fetus with a pH >7.20. The predictive value of a positive test (acceleration with the stimulus) was also 100%, but the predictive value of a negative test (no acceleration with the stimulus) was 8.3%. Similarly, for fetal scalp stimulation, the sensitivity was 43.4%, specificity was 100%, predictive value of a positive test was 100%, and the predictive value of a negative test was 5.5%.

The 39 instances of absent acceleration in response to vibratory acoustic stimulation with a pH >7.25 were examined. There were no differences in gestational age, presence of meconium, indications for fetal pH determination, and variable deceleration occurring with the stimulus compared with the group that responded with an acceleration. Narcotic administration had occurred within 2 hours in 23% of the no acceleration group compared with 10.5% in the acceleration group. This difference was not statistically different.

Comment

The value of fetal vibratory acoustic stimulation during the antepartum period has been previously established. The response to acoustic stimulation has been demonstrated as early as 24 weeks' gestation when studied serially from 12 to 32 weeks.[8] After 28 weeks, an invariant response to acoustic stimulation was noted with notable exceptions. The exceptions noted were in babies with congenital deafness, multiple congenital anomalies, or severe fetal compromise. It has been demonstrated that the normal startle reflex is elicited in response to vibratory acoustic stimulation both in utero and after birth. This reflex indicates an intact nervous system and may change with altered fetal sleep-wake cycles. Vibratory acoustic stimulation has been demonstrated to alter the fetal state of arousal. In this way, vibratory acoustic stimulation has been used to decrease the false-positive rate on the nonstress test by distinguishing between those fetuses who are in a sleep cycle from those who are nonreactive because of fetal compromise.[9, 10]

The value of vibratory acoustic stimulation in the intrapartum period is not as well studied as in the antepartum period.[4, 11] Fetal stimulation to assess acid-base status is not a new concept. This has been done by Clark

et al. by direct tactile scalp stimulation to alter the fetal arousal state. Vibratory acoustic stimulation is better than direct scalp stimulation in that it is less invasive than scalp puncture for pH determination or scalp stimulation alone. The safety of vibratory acoustic stimulation has been addressed by several investigators.[1, 12, 13] Thus far no untoward effects have been noted. Our study assesses the validity of the use of vibratory acoustic stimulation as a predictor of acid-base status in labor.

The specificity of an acceleration in response to vibratory acoustic stimulation is 100% in the nonacidotic fetus. The absence of an acceleration may not indicate fetal compromise, but rather may be caused by an altered state of arousal in the fetus. Although not statistically significant, a higher incidence of patients who received meperidine within 2 hours of stimulation was noted in the group that had no acceleration with a pH >7.25. The larger number of patients studied may partially explain the lack of acceleration in a nonacidotic fetus.

Electronic fetal heart rate monitoring and fetal scalp blood sampling for pH determination are the accepted methods for intrapartum fetal surveillance. Both of these methods have limitations. Fetal heart rate monitoring is complicated by a high false-positive rate.[14, 15] Our study suggests a clinical role for vibratory acoustic stimulation as a noninvasive screening tool for patients with a question of fetal compromise. This proposed approach would be as follows: (1) vibratory acoustic stimulation in the face of abnormal electronic fetal heart rate monitoring, (2) if an acceleration occurs in response to vibratory acoustic stimulation, electronic fetal heart rate monitoring should be continued, and (3) if no acceleration occurs in response to the stimulus, fetal scalp blood pH determination should be performed. Delivery should be considered in those instances of an abnormal fetal heart rate tracing with no response to vibratory acoustic stimulation and an inability to perform a fetal scalp blood pH. This approach could substantially decrease the number of scalp blood pH determinations required while still improving the specificity of electronic fetal heart rate monitoring. Another benefit of vibratory acoustic stimulation is its use in situations in which direct electronic fetal heart rate monitoring or fetal acid-base determination is not technically feasible or contraindicated, such as intact membranes, an unengaged vertex, or lack of cervical dilation.

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Update in Colposcopy and Lower Genital Neoplasia and **Update in Gynecologic Laser Surgery: Advances in Instrumentation and Techniques,** The Breakers, Palm Beach, Florida, February 19 and 20, 1988. For further information contact: Dr. V. C. Wright, Department of Obstetrics and Gynecology, The University of Western Ontario, care of The Women's Clinic, 887 Richmond St., London, Ontario, Canada N6A 3J1. Tel.: (519) 438-1411.

11th Annual Gold Coast OB/GYN Conference, "Current Clinical Concepts in Reproductive Endocrinology and Perinatology," February 8-10, 1988, Good Samaritan Hospital, West Palm Beach, Florida. For more information contact: Laura Lyons, CME Coordinator, Good Samaritan Hospital, P. O. Box 3166, West Palm Beach, FL 33402. Tel.: (305) 650-6236.

High-Risk Obstetrics, March 24-26, 1988, Chicago, Illinois. Sponsored by Cook County Graduate School of Medicine. For additional information, contact the Registrar's Office. Toll-free number: In Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

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Index to volume 157

Author index*

A

Abbott D (*see* Musclow et al). 1987;157:178-9
Abeler VM (*see* Vergote et al). 1987;157:88-92
Abrams RM, Hutchison AA, Gerhardt KJ, Evans SL, Pendergast J. Local cerebral glucose utilization in fetal sheep exposed to noise. 1987;157:456-60
——, ——, McTiernan MJ, Merwin GE. Effects of cochlear ablation on local cerebral glucose utilization in fetal sheep. 1987;157:1438-42
Acker DB, Schulman EB, Ransil BJ, Sachs BP, Friedman EA. The normal parturient's admission temperature. 1987;157:308-11
Ager JW (*see* Poland et al). 1987;157:297-303
Ahn MO, Phelan JP, Smith CV, Jacobs N, Rutherford SE. Antepartum fetal surveillance in the patient with decreased fetal movement. 1987;157:860-4
Aksel S (*see* Prough et al). 1987;157:1449-53
Albu T (*see* Jakobovits et al). 1987;157:1013 (Letter)
Alexander G (*see* Trudinger et al). 1987;157:1443-8
Ali AM (*see* Saleh et al). 1987;157:331-6
Allen AC (*see* Nwaesei et al). 1987;157:890-7
Alpi O (*see* Bonora et al). 1987;157:140-1
Altman R (*see* McGowan et al). 1987;157:726-9
Alvarez JG (*see* Ludmir et al). 1987;157:84-8
Amstey MS, Ogden E. *Haemophilus influenzae* infection and midtrimester abortion. 1987;157:518 (Letter)
Andersen WA, Taylor PT Jr, Fechner RE, Pinkerton JAV. Endometrial metaplasia associated with endometrial adenocarcinoma. 1987;157:597-604
Anderson CF (*see* Reed et al). 1987;157:774-9
—— (*see* Shenker et al). 1987;157:248-53
Anderson GH (*see* Benedet et al). 1987;157:268-73
Anderson MC. Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia. 1987;157:1322 (Letter)
Anderson NC Jr (*see* Bird et al). 1987;157:171-7
Andrew K (*see* Ober et al). 1987;157:1364-8
Ansbacher R (*see* Randolph et al). 1987;157:1534-9
Anteby SO (*see* Margalioth et al). 1987;157:93-6
Anyaegbunam A (*see* Brustman et al). 1987;157:699-703
—— (*see* Fleischer et al). 1987;157:40-3
—— (*see* Langer et al). 1987;157:669-75
—— (*see* Langer et al). 1987;157:758-63
Aoki S (*see* Hata et al). 1987;157:1269-71
Aral SO, Mosher WD, Cates W Jr. Contraceptive use, pelvic inflammatory disease, and fertility problems among American women: 1982. 1987;157:59-64
Archer GE, Furlong LA. Acute abdomen caused by placenta percreta in the second trimester. 1987;157:146-7
Arienzo R, Ricco CS, Romeo F. A very rare fetal malformation: the cutaneous widespread vascular hamartomatosis. 1987;157:1162-3
Arthur M (*see* Devoe et al). 1987;157:240-4
—— (*see* Devoe et al). 1987;157:531-7

*July, 1-228; August, 229-520; September, 521-788; October, part 1, 789-1018; part 2, 1019-1092; November, 1093-1324; December, 1325-1618.

Arulkumaran S, Michelsen J, Ingemarsson I, Ratnam SS. Obstetric outcome of patients with a previous episode of spurious labor. 1987;157:17-20
Ashwal S (*see* Harake et al). 1987;157:1003-8
Atlas SJ (*see* Yoshimura et al). 1987;157:468-75
Auletta FJ, Flint APF. Human ovarian oxytocin and steroid hormones. 1987;157:1010-1 (Letter)
Austin JM Jr (*see* Barter et al). 1987;157:1166-8
Awadalla SG, Freidman CI, Haq AU, Roh SI, Chin NW, Kim MH. Local peritoneal factors: their role in infertility associated with endometriosis. 1987;157:1207-14
Axelson JE (*see* Riggs et al). 1987;157:1286-91
Ayers JWT (*see* Randolph et al). 1987;157:1534-9
Ayyagari RR, Fazleabas AT, Dawood MY. Seminal plasma proteins of fertile and infertile men analyzed by two-dimensional electrophoresis. 1987;157:1528-33

B

Badawy S (*see* Baggish et al). 1987;157:1129-33
Baggish MS. Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia. 1987;157:1323 (Letter reply)
——, Baltoyannis P, Badawy S, Laurey D. Carbon dioxide laser laparoscopy performed with a flexible fiber in humans. 1987;157:1129-33
Bahari C (*see* Brenner et al). 1987;157:1160-1
Bain RP, Greenberg RS, Chung KC. Racial differences in survival of women with endometrial cancer. 1987;157:914-23
Baker DA, Milch PO, Salvatore W, Luft B. Enhancement of maternal and neonatal natural killer cell activity with interleukin-2. 1987;157:780-1
Bakke T (*see* Soernes and Bakke). 1987;157:1229-30
Baldwin RW (*see* Powell et al). 1987;157:28-34
Baltoyannis P (*see* Baggish et al). 1987;157:1129-33
Barbacci M (*see* Quinn et al). 1987;157:394-9
Barkai G (*see* Lipitz et al). 1987;157:1178-81
Barter JF, Soong SJ, Hatch KD, Orr JW Jr, Partridge EC, Austin JM Jr, Shingleton HM. Treatment of nonmetastatic gestational trophoblastic disease with oral methotrexate. 1987;157:1166-8
Barzilai M (*see* Ollendorff et al). 1987;157:71-2
Battaglia FC (*see* Pardi et al). 1987;157:1221-8
Baulieu EE (*see* Garfield et al). 1987;157:1281-5
Baum C (*see* Piper et al). 1987;157:148-56
Bawdon RE (*see* Hemsell et al). 1987;157:498-501
Bean K (*see* Cohen et al). 1987;157:1122-5
Bear MB (*see* Williams et al). 1987;157:708-12
Beaulac-Baillargeon L, Desrosiers C. Caffeine-cigarette interaction on fetal growth. 1987;157:1236-40
Beckmann CRB, Clarke-Pearson DL, Evenhouse R. A reusable plastic training model for teaching Papanicolaou smear technique. 1987;157:259-60
Beier S (*see* Elger et al). 1987;157:1065-74
Bekedam DJ, Visser GHA, Mulder EJH, Poelmann-Weesjes G. Heart rate variation and movement incidence in growth-retarded fetuses: the significance of antenatal late heart rate decelerations. 1987;157:126-33
Belaisch-Allart J (*see* Testart et al). 1987;157:168-71
Belanger K (*see* Lockwood et al). 1987;157:803-8
Benacerraf BR, Frigoletto FD Jr. Soft tissue nuchal fold in the second-trimester fetus: standards for normal measurements compared with those in Down syndrome. 1987;157:1146-9
—— (*see* Lockwood et al). 1987;157:803-8
Benedet JL, Miller DM, Nickerson KG, Anderson GH. The results of cryosurgical treatment of cervical intraepithelial neoplasia at one, five, and ten years. 1987;157:268-73
——, Selke PA, Nickerson KG. Colposcopic evaluation of abnormal Papanicolaou smears in pregnancy. 1987;157:932-7
Benedetti TJ (*see* Luthy et al). 1987;157:676-9
Benirschke K (*see* Lacro et al). 1987;157:833-8
Bennett FC (*see* Luthy et al). 1987;157:676-9
Ben-Noon I (*see* Ollendorff et al). 1987;157:71-2
Bensen J (*see* Nelson et al). 1987;157:572-6
Benson L (*see* Herman et al). 1987;157:1182-3
Bergstralh E (*see* Patton et al). 1987;157:1345-50
Berkeley AS (*see* LaSala and Berkeley). 1987;157:379-83
Berkowitz RL, Chitkara U, Wilkins I, Lynch L, Mehalek KE. Technical aspects of intravascular intrauterine transfusions: lessons learned from thirty-three procedures. 1987;157:4-9 (Clin. opinion)
—— (*see* Wilkins et al). 1987;157:694-8
Bernier LL (*see* Deuster et al). 1987;157:964-8
Bernstein EP (*see* Musclow et al). 1987;157:178-9
Bertrand M, Lickrish GM, Colgan TJ. The anatomic distribution of cervical adenocarcinoma in situ: implications for treatment. 1987;157:21-5
Besinger RE, Compton AA, Hayashi RH. The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor. 1987;157:753-7
Bhatia RK, Bottoms SF, Saleh AA, Norman GS, Mammen EF, Sokol RJ. Mechanisms for reduced colloid osmotic pressure in preeclampsia. 1987;157:106-8
Bhutani VK (*see* Johnson et al). 1987;157:764-9
Biddle PK, Friedman CI, Johnson PM. Lymphocyte-reactive antibodies and recurrent early pregnancy failure. 1987;157:785-6 (Letter)
Billiar RB, Richardson D, Schwartz R, Posner B, Little B. Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys. 1987;157:1297-302
Billings JJ (*see* Brown et al). 1987;157:1082-9
Bird LM, Anderson NC Jr, Chandler ML, Young RC. The effects of aminophylline and nifedipine on contractility of isolated pregnant human myometrium. 1987;157:171-7
Birgerson L (*see* Shoupe et al). 1987;157:1415-20
Blackwell LF (*see* Brown et al). 1987;157:1082-9
Blackwell RE, Garrison PN. Correction of "Inhibition of prolactin secretion by antiserum to the α-and β-subunits of gonadotropin" (1987;156:863-8). 1987;157:1520
Blake D (*see* Grunebaum et al). 1987;157:79-83
Blakemore K (*see* Lockwood et al). 1987;157:803-8
Blankstein J (*see* Geier et al). 1987;157:1009 (Letter)
Blickstein I, Lancet M. Fetal growth retardation as a complication of pregnancy in patients with neurofibromatosis. 1987;157:343
Blodgett WA (*see* Joseph et al). 1987;157:911-2
Bloss JD, Hankins GDV, Hauth JC, Gilstrap LC III. The effect of oxytocin infusion on the pharmacokinetics of intramuscular magnesium sulfate therapy. 1987;157:156-60
Bloxam DL, Bullen BE, Walters BNJ, Lao TT. Placental glycolysis and energy metabolism in preeclampsia. 1987;157:97-101
Bochner CJ, Medearis AL, Davis J, Oakes GK, Hobel CJ, Wade ME. Antepartum predictors of fetal distress in postterm pregnancy. 1987;157:353-8
——, ——, Williams J III, Castro L, Hobel CJ, Wade ME. Early third-trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term. 1987;157:703-8
Boike GM, Gove N, Dombrowski MP, Deppe G, Sokol RJ. Mycotic aneurysms in pregnancy. 1987;157:340-1

Boldt JP (*see* Padilla et al). 1987;157:622-6
Bolognese RJ (*see* Johnson et al). 1987;157:764-9
Bolton V (*see* Hill et al). 1987;157:10-1
Bonnar J. Coagulation effects of oral contraception. 1987;157:1042-8
Bonora E, Zavaroni I, Alpi O, Pezzarossa A, Dall'Aglio E, Coscelli C, Butturini U. Influence of the menstrual cycle on glucose tolerance and insulin secretion. 1987;157:140-1
Boogert A, Mantingh A, Visser GHA. The immediate effects of chorionic villus sampling on fetal movements. 1987;157:137-9
Børmer OP (*see* Vergote et al). 1987;157:88-92
Bors-Koefoed R (*see* Vintzileos et al). 1987;157:236-40
—— (*see* Vintzileos et al). 1987;157:410-4
Bossert NL (*see* Iannaccone et al). 1987;157:476-84 (Curr. dev.)
Bottoms SF (*see* Bhatia et al). 1987;157:106-8
—— (*see* Saleh et al). 1987;157:331-6
Boutselis JG (*see* Rose et al). 1987;157:261-7
Bozzetti P (*see* Pardi et al). 1987;157:1221-8
Bracero L (*see* Ducey et al). 1987;157:680-5
Bracken MB (*see* Shepard et al). 1987;157:217 (Correction)
Bradley L (*see* Trudinger et al). 1987;157:1443-8
Brambati B, Oldrini A, Lanzani A. Transabdominal chorionic villus sampling: a freehand ultrasound-guided technique. 1987;157:134-7
Brar HS, Kjos SL, Dougherty W, Do Y-S, Tam HB, Hsueh WA. Increased fetoplacental active renin production in pregnancy-induced hypertension. 1987;157:363-7
Brashear WT (*see* Kuhnert et al). 1987;157:1241-6
Braverman JJ (*see* Divon et al). 1987;157:1355-8
Brenner B, Shapira A, Bahari C, Haimovich L, Seligsohn U. Hereditary protein C deficiency during pregnancy. 1987;157:1160-1
Brien JF, Clarke DW, Smith GN, Richardson B, Patrick J. Disposition of acute, multiple-dose ethanol in the near-term pregnant ewe. 1987;157:204-11
—— (*see* Richardson et al). 1987;157:1496-502
Britigan BE (*see* Cohen et al). 1987;157:1122-5
Brosens IA, Verleyen A, Cornillie F. The morphologic effect of short-term medical therapy of endometriosis. 1987;157:1215-21
Brown JB, Blackwell LF, Billings JJ, Conway B, Cox RI, Garrett G, Holmes J, Smith MA. Natural family planning. 1987;157:1082-9
Brown KW (*see* Gallup et al). 1987;157:590-6
Brown ZA (*see* Luthy et al). 1987;157:676-9
Brumfield CG, Cloud GA, Finley SC, Cosper P, Davis RO, Huddleston JF. Amniotic fluid α-fetoprotein levels and pregnancy outcome. 1987;157:822-5
Brustman L, Langer O, Engel S, Anyaegbunam A, Mazze R. Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short-and long-term metabolic control in gestational diabetes. 1987;157:699-703
—— (*see* Langer et al). 1987;157:669-75
—— (*see* Langer et al). 1987;157:758-63
Budwit-Novotny DA (*see* Mutch et al). 1987;157:924-31
—— (*see* Soper et al). 1987;157:26-7
Buescher PA (*see* Meis et al). 1987;157:550-6
Bukovsky Y (*see* Herman et al). 1987;157:1182-3
Bullen BE (*see* Bloxam et al). 1987;157:97-101
Burghardt A (*see* Sippo et al). 1987;157:420-1
Burkman RT (*see* Quinn et al). 1987;157:394-9
Burns J (*see* Harper et al). 1987;157:1403-5
Burton BK (*see* Dyer et al). 1987;157:336-9
—— (*see* Nelson et al). 1987;157:572-6
Buscaglia M (*see* Pardi et al). 1987;157:1221-8
Butler WJ (*see* Padilla et al). 1987;157:622-6
Butturini U (*see* Bonora et al). 1987;157:140-1
Byrne JM (*see* Nwaesei et al). 1987;157:890-7

C

Cabbad M, Sijin O, Minkoff H. Short course of antibiotics for post–cesarean section endometritis. 1987;157:908-9
Cabrol D, Landesman R, Muller J, Uzan M, Sureau C, Saxena BB. Treatment of polyhydramnios with prostaglandin synthetase inhibitor (indomethacin). 1987;157:422-6
Callan NA (*see* Johnson et al). 1987;157:764-9
Campbell K (*see* Gagnon et al). 1987;157:743-8
Campbell WA (*see* Rodis et al). 1987;157:1255-7
—— (*see* Vintzileos et al). 1987;157:236-40
—— (*see* Vintzileos et al). 1987;157:410-4
—— (*see* Vintzileos et al). 1987;157:627-31
Campeau JD (*see* Vargyas et al). 1987;157:944-50
Cao J (*see* Liu et al). 1987;157:1079-81
Capeless EL, Mead PB. Management of preterm premature rupture of membranes: lack of a national consensus. 1987;157:11-2
Capetola RJ (*see* Hahn et al). 1987;157:977-82
Cardwell MS. Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage. 1987;157:358-9
Carillo E (*see* Follen et al). 1987;157:809-14
Carlson JR (*see* Hill et al). 1987;157:10-1
Carmichael L (*see* Gagnon et al). 1987;157:1375-81
—— (*see* Richardson et al). 1987;157:1496-502
Carpenter MW (*see* Coustan et al). 1987;157:1113-7
Carpenter RJ Jr (*see* Moise et al). 1987;157:874-9
Carr BR. Metabolism of lipoproteins by human fetal hepatocytes. 1987;157:1338-44
——, Parker CR Jr. The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly. 1987;157:1351-5
Carsten ME, Miller JD. A new look at uterine muscle contraction. 1987;157:1303-15 (Curr. dev.)
Casper RF, Lye SJ. β-Adrenergic receptor agonist infusion increases plasma prostaglandin F levels in pregnant sheep. 1987;157:998-1003
Caspi E (*see* Herman et al). 1987;157:1182-3
Castillo RA, Devoe LD, Falls G, Holzman GB, Hadi HA, Fadel HE. Pleural effusions and pulmonary hypoplasia. 1987;157:1252-5
Castro L (*see* Bochner et al). 1987;157:703-8
—— (*see* Ross et al). 1987;157:1292-7
Catanzarite VA. Risk of vaginal delivery after a prior cesarean section. 1987;157:213 (Letter)
Catella F (*see* Fitzgerald et al). 1987;157:325-30
Cates W Jr (*see* Aral et al). 1987;157:59-64
Cavalieri RL (*see* Guidetti et al). 1987;157:1521-3
Cerreta J (*see* Leppert et al). 1987;157:1134-9
Cetin I (*see* Pardi et al). 1987;157:1221-8
Challis JRG, Vaughan M. Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua. 1987;157:1474-81
—— (*see* Davidson et al). 1987;157:54-8
—— (*see* Mitchell et al). 1987;157:349-53
Chandler ML (*see* Bird et al). 1987;157:171-7
Chang FE (*see* Chin et al). 1987;157:938-44
Chang F-M (*see* Hsieh et al). 1987;157:44-9

Chase JS, Check JH, Nowroozi K, Wu CH. First-trimester serum levels of the β-subunit of human chorionic gonadotropin in a tubal molar pregnancy. 1987;157:910
Chasnoff IJ (*see* MacGregor et al). 1987;157:686-90
Chatterton RT (*see* Kustin et al). 1987;157:1468-73
Chatwani A (*see* Lindenbaum et al). 1987;157:1273-4
Chaudhuri G (*see* Steingold et al). 1987;157:1543-9
Check JH (*see* Chase et al). 1987;157:910
Chen H-Y (*see* Hsieh et al). 1987;157:44-9
Chesley LC, Sibai BM. Blood pressure in the midtrimester and future eclampsia. 1987;157:1258-61
Chestnut DH, Wilcox LL. Influence of umbilical vein administration of oxytocin on the third stage of labor: a randomized, double-blind, placebo-controlled study. 1987;157:160-2
Chi-Lee (*see* Shulman et al). 1987;157:1539-42
Chin NW, Chang FE, Dodds WG, Kim MH, Malarkey WB. Acute effects of exercise on plasma catecholamines in sedentary and athletic women with normal and abnormal menses. 1987;157:938-44
—— (*see* Awadalla et al). 1987;157:1207-14
Chism J (*see* Lehmann and Chism). 1987;157:738-42
Chisum GM (*see* MacGregor et al). 1987;157:686-90
Chitkara U (*see* Berkowitz et al). 1987;157:4-9 (Clin. opinion)
—— (*see* Wilkins et al). 1987;157:694-8
Chorney MC (*see* Nowak et al). 1987;157:464-7
Christensen K, Christensen P. A screening test (GBS-Test) for urogenital carriage of group B streptococci. 1987;157:341-2
Christensen P (*see* Christensen and Christensen). 1987;157:341-2
Christiaens GCML, Helmerhorst FM. Validity of intrapartum diagnosis of fetal thrombocytopenia. 1987;157:864-5
Christiansen E, Veldhuis JD, Rogol AD, Stumpf P, Evans WS. Modulating actions of estradiol on gonadotropin-releasing hormone–stimulated prolactin secretion in postmenopausal individuals. 1987;157:320-5
Chu J, White E. Decreasing incidence of invasive cervical cancer in young women. 1987;157:1105-7
Chung KC (*see* Bain et al). 1987;157:914-23
Chwalisz K (*see* Elger et al). 1987;157:1065-74
Cines DB (*see* Samuels et al). 1987;157:109-13
Clark MR (*see* Curry et al). 1987;157:537-43
Clark SL, Vitale DJ, Minton SD, Stoddard RA, Sabey PL. Successful fetal therapy for cystic adenomatoid malformation associated with second-trimester hydrops. 1987;157:294-5
—— (*see* Phelan et al). 1987;157:1510-5
Clarke DW (*see* Brien et al). 1987;157:204-11
Clarke-Pearson DL (*see* Beckmann et al). 1987;157:259-60
Clement D, Schifrin BS, Kates RB. Acute oligohydramnios in postdate pregnancy. 1987;157:884-6
Clewell WH (*see* Nicolaides et al). 1987;157:50-3
Cloud GA (*see* Brumfield et al). 1987;157:822-5
Cochran EB (*see* Gonzalez et al). 1987;157:660-4
Cohen BH (*see* Khoury and Cohen). 1987;157:400-10
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G

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H

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Mattison DR (*see* Kay et al). 1987;157:185-9
Mayo G (*see* Fitzgerald et al). 1987;157:325-30
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McMorland GH (*see* Riggs et al). 1987;157:1286-91
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McTiernan MJ (*see* Abrams et al). 1987;157:1438-42
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Medearis AL (*see* Bochner et al). 1987;157:353-8
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Schaaps J-P (*see* Hustin and Schaaps). 1987;157:162-8
—— (*see* Hustin and Schaaps). 1987;157:1291 (Correction)
Schachter J (*see* Moscicki et al). 1987;157:65-71
Schaefer TR (*see* Saldana et al). 1987;157:712-5
Schaub RG (*see* Keith et al). 1987;157:199-203
Schenken RS, Williams RF, Hodgen GD. Effect of pregnancy on surgically induced endometriosis in cynomolgus monkeys. 1987;157:1392-6
Schenker JG (*see* Margalioth et al). 1987;157:93-6
Schifman RB, Thomasson JE, Evers JM. Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy. 1987;157:304-7
Schifrin BS (*see* Clement et al). 1987;157:884-6
Schinzel A. Possible vascular disruptive origin of hemifacial microsomia? 1987;157:1319 (Letter)
Schmidt PL (*see* Keegan et al). 1987;157:121-5
Schneider PJ (*see* Fayez and Schneider). 1987;157:1184-8
Schulman EB (*see* Acker et al). 1987;157:308-11
Schulman H (*see* Ducey et al). 1987;157:680-5
—— (*see* Fleischer et al). 1987;157:40-3
Schultz PN (*see* Samaan et al). 1987;157:1524-8
Schwartz R (*see* Billiar et al). 1987;157:1297-302
Scioscia A (*see* Lockwood et al). 1987;157:285-7
Searle N (*see* Devoe et al). 1987;157:240-4
—— (*see* Devoe et al). 1987;157:531-7
—— (*see* McGowan et al). 1987;157:726-9
Segal S. A new delivery system for contraceptive steroids. 1987;157:1090-2
Segre A (*see* Petraglia et al). 1987;157:142-6
Seki K, Kato K. Increased boy-to-girl ratio in women with preterm labor beginning with contractions. 1987;157:215-6 (Letter)
Seligsohn U (*see* Brenner et al). 1987;157:1160-1
Selke PA (*see* Benedet et al). 1987;157:932-7
Senekjian EK, Young JM, Weiser PA, Spencer CE, Magic SE, Herbst AL. An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma. 1987;157:433-9
Shafer M-A (*see* Moscicki et al). 1987;157:65-71
Shalev E, Zuckerman H. Antenatal diagnosis with ultrasonography. 1987;157:1323 (Letter)
Shapira A (*see* Brenner et al). 1987;157:1160-1
Sharma V (*see* Rudelstorfer et al). 1987;157:372-7
Sharp PC (*see* Meis et al). 1987;157:550-6
Shaw P (*see* MacGregor et al). 1987;157:686-90
Shenker L, Reed KL, Anderson CF, Marx GR, Sobonya RE, Graham AR. Congenital heart block and cardiac anomalies in the absence of maternal connective tissue disease. 1987;157:248-53
—— (*see* Reed et al). 1987;157:774-9
Shennan AT (*see* Ohlsson et al). 1987;157:443-5
Shepard MJ, Helenbrand KG, Bracken MB. Correction of "Proportional weight gain and complications of pregnancy, labor, and delivery in healthy women of normal prepregnant stature" (1986;155:947-54). 1987;157:217
Shepherd J (*see* Prough et al). 1987;157:1449-53
Shih C-T (*see* Shinozuka et al). 1987;157:1140-5
Shingleton HM (*see* Barter et al). 1987;157:1166-8
Shinozuka N, Okai T, Kohzuma S, Mukubo M, Shih C-T, Maeda T, Kuwabara Y, Mizuno M. Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes. 1987;157:1140-5
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——, ——, Page MA, Madkour H, Spitz IM, Lobo RA. Effects of the antiprogesterone RU 486 in normal women. II. Administration in the late follicular phase. 1987;157:1421-6
Shulman H, Ducey J, Farmakides G, Guzman E, Winter D, Penny B, Chi-Lee. Uterine artery Doppler velocimetry: the significance of divergent systolic/diastolic ratios. 1987;157:1539-42
Shy KK (*see* Luthy et al). 1987;157:676-9
Sibai BM. HELLP syndrome. 1987;157:1016 (Letter reply)
—— (*see* Chesley and Sibai). 1987;157:1258-61
—— (*see* Gonzalez et al). 1987;157:660-4
—— (*see* Goren et al). 1987;157:906-8
Siegal GP (*see* Moore et al). 1987;157:903-5
Sijin O (*see* Cabbad et al). 1987;157:908-9
Silver RK, Dooley SL, Tamura RK, Depp R. Umbilical cord size and amniotic fluid volume in prolonged pregnancy. 1987;157:716-20
——, Minogue J. When does a statistical fact become an ethical imperative? 1987;157:229-33 (Clin. opinion)
Simbruner G (*see* Rudelstorfer et al). 1987;157:372-7
Sippo WC, Burghardt A, Gomez AC. Nerve entrapment after Pfannenstiel incision. 1987;157:420-1
Smith BC (*see* Miller et al). 1987;157:912-4
Smith BE (*see* Kambam et al). 1987;157:897-9
Smith CV (*see* Ahn et al). 1987;157:860-4
Smith GN (*see* Brien et al). 1987;157:204-11
Smith HO, Toledo AA, Thompson JD. Conservative surgical management of isthmic ectopic pregnancies. 1987;157:604-10

Smith MA (*see* Brown et al). 1987;157:1082-9
Smith RP, Powell JR. Simultaneous objective and subjective evaluation of meclofenamate sodium in the treatment of primary dysmenorrhea. 1987;157:611-8
Smotherman WP, Robinson SR. Stereotypic behavioral response of rat fetuses to acute hypoxia is altered by maternal alcohol consumption. 1987;157:982-6
Sobonya RE (*see* Shenker et al). 1987;157:248-53
Socol ML, MacGregor SN, Pielet BW, Tamura RK, Sabbagha RE. Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops. 1987;157:1369-75
Soernes T, Bakke T. The length of the human umbilical cord in twin pregnancies. 1987;157:1229-30
Sohmer H (*see* Ohel et al). 1987;157:440-1
Sokol RJ (*see* Bhatia et al). 1987;157:106-8
—— (*see* Boike et al). 1987;157:340-1
—— (*see* Wolfe et al). 1987;157:288-93
Soong SJ (*see* Barter et al). 1987;157:1166-8
Soper JT, Cox EB, Budwit-Novotny D, Mutch DG, Creasman WT, McCarty KS Sr, McCarty KS Jr. Histologic composition of endometrial carcinomas analyzed for steroid receptor content. 1987;157:26-7
—— (*see* Mutch et al). 1987;157:924-31
Spanta R, Roffman LE, Grissom TJ, Newland JR, McManus BM. Abdominal pregnancy: magnetic resonance identification with ultrasonographic follow-up of placental involution. 1987;157:887-9
Spellacy WN, Peterson PQ, Winegar A, Quilligan EJ. Neonatal seizures after cesarean delivery: higher risk with labor. 1987;157:377-9
Spence MR (*see* Quinn et al). 1987;157:394-9
Spencer CE (*see* Senekjian et al). 1987;157:433-9
Spencer JW, Cox DN. Emotional responses of pregnant women to chorionic villi sampling or amniocentesis. 1987;157:1155-60
Speroff L. International symposium on contraception. Part I: oral contraception: introduction. 1987;157:1019
Spitz IM (*see* Shoupe et al). 1987;157:1415-20
—— (*see* Shoupe et al). 1987;157:1421-6
Stanczyk FZ (*see* Haluska et al). 1987;157:1487-95
Steingold KA, Pardridge WM, Judd HL, Chaudhuri G. The effects of membrane permeability and binding by human serum proteins on steroid influx into the rabbit uterus. 1987;157:1543-9
Stenman U-H (*see* Ylikorkala et al). 1987;157:344-8
Stevens D (*see* Trudinger et al). 1987;157:1443-8
Stewart DE, Raskin J, Garfinkel PE, MacDonald OL, Robinson GE. Anorexia nervosa, bulimia, and pregnancy. 1987;157:1194-8
Stiller R (*see* Lockwood et al). 1987;157:285-7
Stinson DA (*see* Nwaesei et al). 1987;157:890-7
Stockwell HG, Lyman GH. Cigarette smoking and the risk of female reproductive cancer. 1987;157:35-40
Stoddard RA (*see* Clark et al). 1987;157:294-5
Storchi R (*see* Petraglia et al). 1987;157:142-6
Strickland D (*see* Luthy et al). 1987;157:676-9
Strickland DM, Hankins GDV. Amniotic fluid discoloration caused by blood. 1987;157:216-7 (Letter)
Strobino BA, Pantel-Silverman J. First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions. 1987;157:1150-4
Stuart MJ (*see* Kuhn and Stuart). 1987;157:194-8
Stumpf P (*see* Christiansen et al). 1987;157:320-5
Sureau C (*see* Cabrol et al). 1987;157:422-6
Surti U (*see* Vejerslev et al). 1987;157:180-4 (Curr. dev.)
Swaab DF (*see* Oosterbaan and Swaab). 1987;157:117-9
Swain M (*see* Harper et al). 1987;157:1403-5
Symonds EM (*see* Powell et al). 1987;157:28-34
Syracuse CD (*see* Philipson et al). 1987;157:1275-8
Szalay S (*see* Petraglia et al). 1987;157:142-6
Szewczyk K (*see* Devaskar et al). 1987;157:460-4

T

Takagi S (*see* Sakamoto et al). 1987;157:318-9
Takahashi Y (*see* Nimrod et al). 1987;157:655-9
Takamiya O (*see* Hata et al). 1987;157:1269-71
Takamizawa M, Juji T, Tsuneyoshi H, Nieda M, Fujii T, Kawana T, Mizuno M. Recurrent spontaneous abortion and human leukocyte antigen DRw8. 1987;157:514-5 (Letter)
Talledo OE (*see* Gallup et al). 1987;157:590-6
Talwar GP, Gaur A. Recent developments in immunocontraception. 1987;157:1075-8
Tam HB (*see* Brar et al). 1987;157:363-7
Tamura RK (*see* Pielet et al). 1987;157:1411-4
—— (*see* Silver et al). 1987;157:716-20
—— (*see* Socol et al). 1987;157:1369-75
Tang L (*see* Muechler et al). 1987;157:453-5
Taylor PT Jr (*see* Andersen et al). 1987;157:597-604
Taylor SM (*see* Riggs et al). 1987;157:1286-91
ten Hove W (*see* Iffy et al). 1987;157:516 (Letter reply)
Tennant FR (*see* Morris et al). 1987;157:857-8
Testart J, Lassalle B, Belaisch-Allart J, Forman R, Hazout A, Volante M, Frydman R. Human embryo viability related to freezing and thawing procedures. 1987;157:168-71
Thacker SB. Intrapartum electronic fetal monitoring efficacy. 1987;157:1014 (Letter reply)
Thatcher CD (*see* Keith et al). 1987;157:199-203
Thejls H, Rahm VA, Rosen G, Gnarpe H. Correlation between chlamydia infection and clinical evaluation, vaginal wet smear, and cervical swab test in female adolescents. 1987;157:974-6
Thiede HA, Saini VD. Urogynecology: comments and caveats. 1987;157:563-8
Thomasson JE (*see* Schifman et al). 1987;157:304-7
Thompson JD (*see* Smith et al). 1987;157:604-10
Thompson JS (*see* Ducsay et al). 1987;157:1482-6
Thompson RS (*see* Trudinger et al). 1987;157:1443-8
Thompson SC (*see* Graham et al). 1987;157:254-7
Tho SPT (*see* Padilla et al). 1987;157:622-6
Thurnau GR, Kemp DB, Jarvis A. Cerebrospinal fluid levels of magnesium in patients with preeclampsia after treatment with intravenous magnesium sulfate: a preliminary report. 1987;157:1435-8
Tice T (*see* Erskine et al). 1987;157:213 (Letter reply)
Tihansky DP, DiBlasio JA. Breast galactography in the treatment of infected cysts. 1987;157:1278-80
Timor-Tritsch IE, Yeh M-N. In vitro training model for diagnostic and therapeutic fetal intravascular needle puncture. 1987;157:858-9
Tokushige M (*see* Wang et al). 1987;157:956-63
Toledo AA (*see* Smith et al). 1987;157:604-10
Tomaski A (*see* Samuels et al). 1987;157:109-13
Touchstone JC (*see* Ludmir et al). 1987;157:84-8
Towell ME, Figueroa J, Markowitz S, Elias B, Nathanielsz P. The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation. 1987;157:1550-7
Trostmann UH (*see* Deuster et al). 1987;157:964-8
Trudinger BJ, Stevens D, Connelly A, Hales JRS, Alexander G, Bradley L, Fawcett A, Thompson RS. Umbilical artery

flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation. 1987;157:1443-8
Truong CT (*see* Hoffman et al). 1987;157:179 (Correction)
Trujillo R (*see* Elliott and Trujillo). 1987;157:245-7
Tsubata K (*see* Sakamoto et al). 1987;157:318-9
Tsuneyoshi H (*see* Takamizawa et al). 1987;157:514-5 (Letter)
Tyner J (*see* Higuchi et al). 1987;157:986-90

U

Udassin R (*see* Margalioth et al). 1987;157:93-6
Uzan M (*see* Cabrol et al). 1987;157:422-6

V

Vale W (*see* Liu et al). 1987;157:1387-91
Valenzuela GJ (*see* Davidson et al). 1987;157:54-8
Vargyas JM, Campeau JD, Mishell DR Jr. Treatment of menorrhagia with meclofenamate sodium. 1987;157:944-50
Vaughan M (*see* Challis and Vaughan). 1987;157:1474-81
Veiga-Ferreira MM, Leiman G, Dunbar F, Margolius KA. Cervical endometriosis: facilitated diagnosis by fine needle aspiration cytologic testing. 1987;157:849-56
Vejerslev LO, Fisher RA, Surti U, Walke N. Hydatidiform mole: cytogenetically unusual cases and their implications for the present classification. 1987;157:180-4 (Curr. dev.)
Veldhuis JD (*see* Christiansen et al). 1987;157:320-5
Vergote IB, Børmer OP, Abeler VM. Evaluation of serum CA 125 levels in the monitoring of ovarian cancer. 1987;157:88-92
Verleyen A (*see* Brosens et al). 1987;157:1215-21
Vermesh M, Kletzky OA. Follicle-stimulating hormone is the main determinant of follicular recruitment and development in ovulation induction with human menopausal gonadotropin. 1987;157:1397-402
—— (*see* Rosen et al). 1987;157:1272-3
Viechnicki BM (*see* Patriarco et al). 1987;157:384-7
Vincer MJ (*see* Nwaesei et al). 1987;157:890-7
Vintzileos AM, Bors-Koefoed R, Pelegano JF, Campbell WA, Rodis JF, Nochimson DJ, Kontopoulos VG. The use of fetal biophysical profile improves pregnancy outcome in premature rupture of the membranes. 1987;157:236-40
——, Campbell WA, Rodis JF, Bors-Koefoed R, Nochimson DJ. Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements. 1987;157:410-4
——, Gaffney SE, Salinger LM, Kontopoulos VG, Campbell WA, Nochimson DJ. The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores. 1987;157:627-31
—— (*see* Rodis et al). 1987;157:1255-7
Visser GHA (*see* Bekedam et al). 1987;157:126-33
—— (*see* Boogert et al). 1987;157:137-9
Vitale DJ (*see* Clark et al). 1987;157:294-5
Volante M (*see* Testart et al). 1987;157:168-71
Volpe A (*see* Petraglia et al). 1987;157:142-6

W

Wachtel S (*see* Rosen et al). 1987;157:1272-3
Wade ME (*see* Bochner et al). 1987;157:353-8
—— (*see* Bochner et al). 1987;157:703-8
Waeltz JL (*see* Jones et al). 1987;157:544-9
Walke N (*see* Vejerslev et al). 1987;157:180-4 (Curr. dev.)
Wallach EE (*see* Yoshimura et al). 1987;157:468-75
Wallenburg HCS, Rotmans N. Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole. 1987;157:1230-5
Walters BNJ (*see* Bloxam et al). 1987;157:97-101
Walton LA (*see* Moore et al). 1987;157:903-5
Wan M (*see* Romero et al). 1987;157:1454-60
—— (*see* Romero et al). 1987;157:1461-7
Wang H-S, Kanzaki H, Yoshida M, Sato S, Tokushige M, Mori T. Suppression of lymphocyte reactivity in vitro by supernatants of explants of human endometrium. 1987;157:956-63
Washburn NR. Screening for genetic anomalies. 1987;157:212 (Letter)
Wason CJ (*see* Ober et al). 1987;157:1364-8
Wastie ML (*see* Powell et al). 1987;157:28-34
Way DL (*see* David et al). 1987;157:969-73
Webster S (*see* Kuhnert et al). 1987;157:1241-6
Weiner CP, Landas S, Persoon TJ. Digoxin-like immunoreactive substance in fetuses with and without cardiac pathology. 1987;157:368-71
Weiner S (*see* Johnson et al). 1987;157:764-9
Weiner SA (*see* David et al). 1987;157:969-73
Weingold AB. Presidential address of Association of Professors of Gynecology and Obstetrics. 1987;157:799-802
Weinraub Z (*see* Herman et al). 1987;157:1182-3
Weiser PA (*see* Senekjian et al). 1987;157:433-9
Welch RA (*see* Saleh et al). 1987;157:331-6
West LC (*see* Iams et al). 1987;157:638-43
White E (*see* Chu and White). 1987;157:1105-7
Widness JA (*see* Coustan et al). 1987;157:1113-7
Wiebe H (*see* Prough et al). 1987;157:1449-53
Wilcox LL (*see* Chestnut and Wilcox). 1987;157:160-2
Wilkins IA, Chitkara U, Lynch L, Goldberg JD, Mehalek KE, Berkowitz RL. The nonpredictive value of fetal urinary electrolytes: preliminary report of outcomes and correlation with pathologic diagnosis. 1987;157:694-8
—— (*see* Berkowitz et al). 1987;157:4-9 (Clin. opinion)
Williams J III, Medearis AL, Bear MB, Kaback MM. Chorionic villus sampling is associated with normal fetal growth. 1987;157:708-12
—— (*see* Bochner et al). 1987;157:703-8
Williams RF (*see* Schenken et al). 1987;157:1392-6
Williams TG (*see* Kuhnert et al). 1987;157:1247-51
Willis DC. Criteria for interpretation of a reactive nonstress test. 1987;157:213 (Letter)
Wilson J (*see* Luthy et al). 1987;157:676-9
Winegar A (*see* Spellacy et al). 1987;157:377-9
Winkel CA (*see* Hoskins et al). 1987;157:730-2
—— (*see* Hoskins et al). 1987;157:770-3
Winter D (*see* Ducey et al). 1987;157:680-5
—— (*see* Shulman et al). 1987;157:1539-42
Wolfe HM, Gross TL, Sokol RJ. Recurrent small for gestational age birth: perinatal risks and outcomes. 1987;157:288-93
Woods JR Jr (*see* Plessinger and Woods). 1987;157:1382-7
Woosley JT (*see* Moore et al). 1987;157:903-5
Wright JN (*see* Hollander et al). 1987;157:839-42
Wright L (*see* Hollander et al). 1987;157:839-42
Wu AT (*see* Ducsay et al). 1987;157:1482-6
Wu CH (*see* Chase et al). 1987;157:910

Y

Yagel S, Zacut D, Igelstein S, Palti Z, Hurwitz A, Rosenn B. In utero ponderal index as a prognostic factor in the evaluation of intrauterine growth retardation. 1987;157:415-9
Yeh M-N (*see* Timor-Tritsch and Yeh). 1987;157:858-9
Yeh S-Y (*see* Patriarco et al). 1987;157:384-7
Yen SSC (*see* Liu et al). 1987;157:1387-91

Ylikorkala O, Stenman U-H, Halmesmäki E. γ-Glutamyl transferase and mean cell volume reveal maternal alcohol abuse and fetal alcohol effects. 1987;157:344-8
Yonekura ML (*see* Graham et al). 1987;157:254-7
Yoo SD (*see* Riggs et al). 1987;157:1286-91
Yoshida M (*see* Wang et al). 1987;157:956-63
Yoshimura Y, Santulli R, Atlas SJ, Fujii S, Wallach EE. The effects of proteolytic enzymes on in vitro ovulation in the rabbit. 1987;157:468-75
Young BK (*see* Kirshenbaum et al). 1987;157:748-52
Young DC (*see* Nwaesei et al). 1987;157:890-7
Young JM (*see* Senekjian et al). 1987;157:433-9
Young RC (*see* Bird et al). 1987;157:171-7
Younis JS, Granat M. Insufficient transplacental digoxin transfer in severe hydrops fetalis. 1987;157:1268-9
Yu SY (*see* Leppert et al). 1987;157:1134-9

Z

Zacut D (*see* Yagel et al). 1987;157:415-9
Zaltz A (*see* Erskine et al). 1987;157:213 (Letter reply)
Zavaroni I (*see* Bonora et al). 1987;157:140-1
Zongrone J (*see* Muechler et al). 1987;157:1126-8
Zorn EM (*see* Greve et al). 1987;157:217 (Letter reply)
Zubek L (*see* Jakobovits et al). 1987;157:1013 (Letter)
Zuckerman H (*see* Shalev and Zuckerman). 1987;157:1323 (Letter)

Subject index*

A

Abdomen, acute
Acute abdomen caused by placenta percreta in the second trimester (Archer and Furlong). 1987;157:146-7

Abdominal circumference
Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements (Vintzileos et al). 1987;157:410-4
Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia (Johnson et al). 1987;157:764-9

Abdominal hysterectomy
Single-dose prophylaxis for vaginal and abdominal hysterectomy (Hemsell et al). 1987;157:498-501

Abdominal pregnancy
Abdominal pregnancy: magnetic resonance identification with ultrasonographic follow-up of placental involution (Spanta et al). 1987;157:887-9

Abortion
Femininity—a problem(?) (Eichner) (Letter). 1987;157:517
First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions (Strobino and Pantel-Silverman). 1987;157:1150-4
Haemophilus influenzae infection and midtrimester abortion (Amstey and Ogden) (Letter). 1987;157:518
Lymphocyte-reactive antibodies and recurrent early pregnancy failure (Biddle et al) (Letter). 1987;157:785-6
Morality of induced abortion and freedom of choice (Fribourg) (Letter); (Hill) (Reply). 1987;157:215
Recurrent spontaneous abortion and human leukocyte antigen DRw8 (Takamizawa et al) (Letter). 1987;157:514-5

Abruptio placentae
Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage (Cardwell). 1987;157:358-9

Acetaldehyde
Disposition of acute, multiple-dose ethanol in the near-term pregnant ewe (Brien et al). 1987;157:204-11

N-Acetyl-β-D-glucosaminidase
Increased tubular enzyme excretion in preeclampsia (Goren et al). 1987;157:906-8

Acid-base balance; *see also* **Acidosis**
Cord sampling for the evaluation of oxygenation and acid-base balance in human growth-retarded fetuses (Pardi et al). 1987;157:1221-8
Effects of maternal glucose infusion on fetal acid-base status in human pregnancy (Philipson et al). 1987;157:866-73
Indicators of perinatal asphyxia (Hollander et al). 1987;157:839-42

Acidosis; *see also* **Acid-base balance**
The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores (Vintzileos et al). 1987;157:627-31
Umbilical cord blood pH and Apgar score as an index of neonatal health (Josten et al). 1987;157:843-8

Acoustic stimulation
Antepartum fetal surveillance in the patient with decreased fetal movement (Ahn et al). 1987;157:860-4
Fetal auditory brain stem response: effect of increasing stimulus rate during functional auditory development (Plessinger and Woods). 1987;157:1382-7
Fetal heart rate response to vibratory acoustic stimulation predicts fetal pH in labor (Edersheim et al). 1987;157:1557-60
Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term (Gagnon et al). 1987;157:1375-81
Intrapartum vibratory acoustic stimulation of the human fetus during episodes of decreased heart rate variability (Divon et al). 1987;157:1355-8
Neonatal auditory acuity following in utero vibratory acoustic stimulation (Ohel et al). 1987;157:440-1

Acquired immunodeficiency syndrome
Isolation of acquired immunodeficiency syndrome virus from the placenta (Hill et al). 1987;157:10-1

Actin-myosin interaction
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)

Acute abdomen
Acute abdomen caused by placenta percreta in the second trimester (Archer and Furlong). 1987;157:146-7

Adenocarcinoma
The anatomic distribution of cervical adenocarcinoma in situ: implications for treatment (Bertrand et al). 1987;157:21-5
Endometrial adenocarcinoma estrogen receptor content: association of clinicopathologic features with immunohistochemical analysis compared with standard biochemical methods (Mutch et al). 1987;157:924-31
Endometrial metaplasia associated with endometrial adenocarcinoma (Andersen et al). 1987;157:597-604

Adenomatoid malformation
Successful fetal therapy for cystic adenomatoid malformation associated with second-trimester hydrops (Clark et al). 1987;157:294-5

Adenosine monophosphate
Changes in cyclic adenosine monophosphate–phosphodiesterase activity in nonpregnant and pregnant human myometrium (Kofinas et al). 1987;157:733-8

Adenosquamous carcinoma
Adenosquamous carcinoma of the fallopian tube: a clinicopathologic case report with verification of the diagnosis by immunohistochemical and ultrastructural studies (Moore et al). 1987;157:903-5

*July, 1-228; August, 229-520; September, 521-788; October, part 1, 789-1018; part 2, 1019-1092; November, 1093-1324; December, 1325-1618.

Adhesion
Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8
Adnexal cyst
Serum CA-125 in the evaluation of benign adnexal cysts (Pittaway et al). 1987;157:1426-8
Adolescence
Correlation between chlamydia infection and clinical evaluation, vaginal wet smear, and cervical swab test in female adolescents (Thejls et al). 1987;157:974-6
Teen pregnancies (Roush) (Letter); (Hollingsworth) (Reply). 1987;157:1011
The use and limitations of endocervical Gram stains and mucopurulent cervicitis as predictors for *Chlamydia trachomatis* in female adolescents (Moscicki et al). 1987;157:65-71
β-Adrenergic receptor agonist
β-Adrenergic receptor agonist infusion increases plasma prostaglandin F levels in pregnant sheep (Casper and Lye). 1987;157:998-1003
Aerobic conditioning
Effects of aerobic and strength conditioning on pregnancy outcomes (Hall and Kaufmann). 1987;157:1199-203
Age factors
Menstrual and reproductive factors and the risk of myocardial infarction in women under fifty-five years of age (La Vecchia et al). 1987;157:1108-12
Patterns of human fetal heart rate accelerations from 26 weeks to term (Gagnon et al). 1987;157:743-8
Pregnancy outcome in medically complicated and uncomplicated patients aged 40 years or older (Lehmann and Chism). 1987;157:738-42
Aging
Age-related changes in the female hormonal environment during reproductive life (Musey et al). 1987;157:312-7
AIDS; *see* **Acquired immunodeficiency syndrome**
Alanine aminopeptidase
Increased tubular enzyme excretion in preeclampsia (Goren et al). 1987;157:906-8
Alcohol; *see also* **Ethanol**
Cerebral oxidative metabolism in fetal sheep with multiple-dose ethanol infusion (Richardson et al). 1987;157:1496-502
γ-Glutamyl transferase and mean cell volume reveal maternal alcohol abuse and fetal alcohol effects (Ylikorkala et al). 1987;157:344-8
Stereotypic behavioral response of rat fetuses to acute hypoxia is altered by maternal alcohol consumption (Smotherman and Robinson). 1987;157:982-6
Ambulation
The effects of maternal ambulation on the nonstress test (Devoe et al). 1987;157:240-4
The American Board of Obstetrics and Gynecology
Approved fellowship training programs in obstetrics and gynecology subspecialties; Gynecologic Oncology; Reproductive Endocrinology; Maternal-Fetal Medicine. 1987;157:30A-5A
New certifications: subspecialty of Basic. 1987;157:218-24
New certifications: subspecialty of Gynecologic Oncology. 1987;157:226
New certifications: subspecialty of Maternal-Fetal Medicine. 1987;157:224-5
New certifications: subspecialty of Reproductive Endocrinology. 1987;157:225-6
Aminophylline
The effects of aminophylline and nifedipine on contractility of isolated pregnant human myometrium (Bird et al). 1987;157:171-7
Amniocentesis; *see also* **Amniotic fluid**
Emotional responses of pregnant women to chorionic villi sampling or amniocentesis (Spencer and Cox). 1987;157:1155-60
Amnion
Progesterone synthesis by human amnion, chorion, and decidua at term (Mitchell et al). 1987;157:349-53
Amniotic fluid
Amniotic fluid discoloration caused by blood (Strickland and Hankins) (Letter); (Greve et al) (Reply). 1987;157:216-7
Amniotic fluid α-fetoprotein levels and pregnancy outcome (Brumfield et al). 1987;157:822-5
Amniotic fluid trypsin and premature rupture of membranes (McGregor et al) (Letter); (Kanayama) (Reply). 1987;157:1316-9
Antepartum fetal surveillance in the patient with decreased fetal movement (Ahn et al). 1987;157:860-4
Antepartum predictors of fetal distress in postterm pregnancy (Bochner et al). 1987;157:353-8
Arachidonate lipoxygenase metabolites in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1454-60
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91
Effects of alterations of zinc-to-phosphorus ratios and meconium content on Group B *Streptococcus* growth in human amniotic fluid in vitro (Hoskins et al). 1987;157:770-3
Infection and labor: the detection of endotoxin in amniotic fluid (Romero et al). 1987;157:815-9
Leukocyte esterase activity in human amniotic fluid for the rapid detection of chorioamnionitis (Hoskins et al). 1987;157:730-2
Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1461-7
Sonographic amniotic fluid measurement and fetal growth retardation: a reappraisal (Patterson et al). 1987;157:1406-10
Umbilical cord size and amniotic fluid volume in prolonged pregnancy (Silver et al). 1987;157:716-20
Androgens
Age-related changes in the female hormonal environment during reproductive life (Musey et al). 1987;157:312-7
Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys (Billiar et al). 1987;157:1297-302
Anemia
Intrapartum use of fetal scalp hematocrit in the diagnosis of profound fetal anemia caused by fetomaternal hemorrhage (Herman et al). 1987;157:1182-3
Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy (Schifman et al). 1987;157:304-7
Anencephaly
The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly (Carr and Parker). 1987;157:1351-5
Circulating neurohypophyseal hormones in anencephalic infants (Oosterbaan and Swaab). 1987;157:117-9

Aneurysm
Mycotic aneurysms in pregnancy (Boike et al). 1987;157:340-1
Anomaly
Congenital heart block and cardiac anomalies in the absence of maternal connective tissue disease (Shenker et al). 1987;157:248-53
The detection of facial anomalies with ultrasound (Koontz) (Letter); (Pilu) (Reply). 1987;157:1316
Genetic implications of idiopathic hydramnios (Landy et al). 1987;157:114-7
Hypoglycemia: How little is too much for the embryo? (Sadler and Hunter). 1987;157:190-3
A hypothesis concerning the general basis of organogenetic congenital anomalies (Naftolin et al). 1987;157:1-4 (Clin. opinion)
Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984 (Ohlsson et al). 1987;157:443-5
Screening for genetic anomalies (Washburn) (Letter); (Macri) (Reply). 1987;157:212-3
Successful fetal therapy for cystic adenomatoid malformation associated with second-trimester hydrops (Clark et al). 1987;157:294-5
A very rare fetal malformation: the cutaneous widespread vascular hamartomatosis (Arienzo et al). 1987;157:1162-3
Anorexia nervosa
Anorexia nervosa, bulimia, and pregnancy (Stewart et al). 1987;157:1194-8
Antepartum hemorrhage
Fetal-induced trauma as a cause of antepartum hemorrhage (Eden). 1987;157:830-1
Antepartum testing
Antenatal diagnosis and management of monoamniotic twins (Rodis et al). 1987;157:1255-7
Antenatal diagnosis with ultrasonography (Shalev and Zuckerman) (Letter). 1987;157:1323
Antepartum fetal surveillance in the patient with decreased fetal movement (Ahn et al). 1987;157:860-4
Antepartum predictors of fetal distress in postterm pregnancy (Bochner et al). 1987;157:353-8
Antibiotics
Changing concepts in obstetric antibiotic prophylaxis (Galask). 1987;157:491-7
Cost-effective use of antibiotic prophylaxis for cesarean section (Ford et al). 1987;157:506-10
Prophylactic antibiotics for cesarean delivery: a simple cost-effective strategy for prevention of postoperative morbidity (Duff). 1987;157:794-8 (Clin. opinion)
Short course of antibiotics for post–cesarean section endometritis (Cabbad et al). 1987;157:908-9
Antibody
Diagnostic imaging of gynecologic tumors with the monoclonal antibody 791T/36 (Powell et al). 1987;157:28-34
Lymphocyte-reactive antibodies and recurrent early pregnancy failure (Biddle et al) (Letter). 1987;157:785-6
A pregnant woman with a high level of naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii* (Konishi). 1987;157:832-3
The prevalence of autoantibodies in pregnant and nonpregnant women (Patton et al). 1987;157:1345-50
Recent developments in immunocontraception (Talwar and Gaur). 1987;157:1075-8
Varicella zoster antibody testing in the care of pregnant women exposed to varicella (McGregor et al). 1987;157:281-4
Anticoagulant
Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant (Druzin et al). 1987;157:1503-10
Antiemetic
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91
Antigen
An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma (Senekjian et al). 1987;157:433-9
HLA antigen expression and induction by γ-interferon in cultured human trophoblasts (Feinman et al). 1987;157:1429-34
Human leukocyte antigen associated with endometrial carcinoma with a new classification of endometrial carcinoma based on its etiology (Kanuma and Igarashi). 1987;157:427-32
Recurrent spontaneous abortion and human leukocyte antigen DRw8 (Takamizawa et al) (Letter). 1987;157:514-5
Antiglobulin
Abnormalities in platelet antiglobulin tests in preeclamptic mothers and their neonates (Samuels et al). 1987;157:109-13
Antihistamine
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91
Antiplasmin
Preeclampsia, delivery, and the hemostatic system (Saleh et al). 1987;157:331-6
Antiprogesterone
Effects of the antiprogesterone RU 486 in normal women. I. Single-dose administration in the midluteal phase (Shoupe et al). 1987;157:1415-20
Effects of the antiprogesterone RU 486 in normal women. II. Administration in the late follicular phase (Shoupe et al). 1987;157:1421-6
Effects of the antiprogesterone RU 486 on preterm birth in the rat (Garfield et al). 1987;157:1281-5
Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs (Elger et al). 1987;157:1065-74
Temporal changes in uterine activity and prostaglandin response to RU 486 in rhesus macaques in late gestation (Haluska et al). 1987;157:1487-95
Antithrombin
Preeclampsia, delivery, and the hemostatic system (Saleh et al). 1987;157:331-6
Antithyroid drugs
Antithyroid drugs: to breast-feed or not to breast-feed (Cooper). 1987;157:234-5 (Clin. opinion)
Apgar score
The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores (Vintzileos et al). 1987;157:627-31
Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9
Umbilical cord blood pH and Apgar score as an index of neonatal health (Josten et al). 1987;157:843-8
Arachidonate lipoxygenase
Arachidonate lipoxygenase metabolites in amniotic fluid of

women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1454-60

Areoli
Breast galactography in the treatment of infected cysts (Tihansky and DiBlasio). 1987;157:1278-80

Arginine vasopressin
The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation (Towell et al). 1987;157:1550-7

Aromatase
The effect of insulin on aromatase activity in isolated human endometrial glands and stroma (Randolph et al). 1987;157:1534-9

Arrhythmias; *see also* **Tachycardia**
Digoxin-like immunoreactive substance in fetuses with and without cardiac pathology (Weiner et al). 1987;157:368-71

Asphyxia
Indicators of perinatal asphyxia (Hollander et al). 1987;157:839-42
Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984 (Ohlsson et al). 1987;157:443-5

Aspiration biopsy; *see* **Biopsy**

Aspirin
Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole (Wallenburg and Rotmans). 1987;157:1230-5

Association of Professors of Gynecology and Obstetrics
Presidential address of Association of Professors of Gynecology and Obstetrics (Weingold). 1987;157:799-802

Atherosis
Acute atherosis in preeclampsia: maternal determinants and fetal outcome in the presence of the lesion (Khong et al). 1987;157:360-3

Atrial natriuretic peptide
Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus (Ross et al). 1987;157:1292-7

Auditory perception; *see also* **Acoustic stimulation**
Neonatal auditory acuity following in utero vibratory acoustic stimulation (Ohel et al). 1987;157:440-1
Fetal auditory brain stem response: effect of increasing stimulus rate during functional auditory development (Plessinger and Woods). 1987;157:1382-7

Autoantibody
The prevalence of autoantibodies in pregnant and nonpregnant women (Patton et al). 1987;157:1345-50

Autosome 13
A novel Y/13 familial translocation (Morris et al). 1987;157:857-8

B

Bacteria; *see also* **Infection**
Necrotizing fasciitis of the vulva (Roberts). 1987;157:568-71
The use and limitations of endocervical Gram stains and mucopurulent cervicitis as predictors for *Chlamydia trachomatis* in female adolescents (Moscicki et al). 1987;157:65-71

Bart's hemoglobin
Percutaneous ultrasound-guided fetal blood sampling in the management of nonimmune hydrops fetalis (Hsieh et al). 1987;157:44-9

Bereavement
Evaluation of a perinatal grief support team (Lake et al). 1987;157:1203-6

Biophysical profile
Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths (Manning et al). 1987;157:880-4
The use of fetal biophysical profile improves pregnancy outcome in premature rupture of the membranes (Vintzileos et al). 1987;157:236-40

Biopsy
Cervical endometriosis: facilitated diagnosis by fine needle aspiration cytologic testing (Veiga-Ferreira et al). 1987;157:849-56
Placental bed biopsy technique and vascular lesions (Labarrere) (Letter); (Robertson) (Reply). 1987;157:1320-2

Biparietal diameter
A sonographic screening method for Down syndrome (Lockwood et al). 1987;157:803-8

Birth; *see* **Delivery**

Birth weight
Growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels compared in premature, small, average birth weight, and large infants (Samaan et al). 1987;157:1524-8
The relationship between cadmium, zinc, and birth weight in pregnant women who smoke (Kuhnert et al). 1987;157:1247-51
Ultrasonic prediction of birth weight in preterm fetuses: Which formula is best? (Pielet et al). 1987;157:1411-4

Black gravid women
Maternal serum α-fetoprotein screening. III. Pitfalls in evaluating black gravid women (Macri et al). 1987;157:820-2

Bladder polyp
Sonographic diagnosis of a bladder polyp (Jensen and Lynne). 1987;157:1168-9

Bleeding; *see also* **Hemorrhage**
First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions (Strobino and Pantel-Silverman). 1987;157:1150-4

Blood
Amniotic fluid discoloration caused by blood (Strickland and Hankins) (Letter); (Greve et al) (Reply). 1987;157:216-7
Riboflavin concentration in maternal and cord blood in human pregnancy (Kirshenbaum et al). 1987;157:748-52

Blood flow
Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow (Reed et al). 1987;157:774-9
Umbilical blood waveforms in fetal surveillance of twins (Saldana et al). 1987;157:712-5

Blood pressure; *see also* **Hypertension**
Blood pressure in the midtrimester and future eclampsia (Chesley and Sibai). 1987;157:1258-61
Vasopressin-induced bradycardia in fetal and adult sheep is not dependent on an increase in blood pressure (Harper and Rose). 1987;157:448-53

Blood sampling; *see also* **Cord blood**
Percutaneous ultrasound-guided fetal blood sampling in the management of nonimmune hydrops fetalis (Hsieh et al). 1987;157:44-9
Validity of intrapartum diagnosis of fetal thrombocytopenia (Christiaens and Helmerhorst). 1987;157:864-5

Blood volume
Measurement of human fetoplacental blood volume in erythroblastosis fetalis (Nicolaides et al). 1987;157:50-3
Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus (Ross et al). 1987;157:1292-7

Books received
Books received. 1987;157:227, 519, 787, 1017, 1561
Bowel obstruction
Mechanical intestinal obstruction in patients with gynecologic disease: a review of 368 patients (Krebs and Goplerud). 1987;157:577-83
Bradycardia
Vasopressin-induced bradycardia in fetal and adult sheep is not dependent on an increase in blood pressure (Harper and Rose). 1987;157:448-53
Brain death
The pregnant brain dead and the fetus: Must we always try to wrest life from death? (Loewy). 1987;157:1097-101 (Clin. opinion)
Breast
Breast galactography in the treatment of infected cysts (Tihansky and DiBlasio). 1987;157:1278-80
Breast stimulation test and oxytocin challenge test in fetal surveillance: a prospective randomized study (Lipitz et al). 1987;157:1178-81
Cigarette smoking and the risk of female reproductive cancer (Stockwell and Lyman). 1987;157:35-40
Breast-feeding
Antithyroid drugs: to breast-feed or not to breast-feed (Cooper). 1987;157:234-5 (Clin. opinion)
Mexiletine use in pregnancy and lactation (Lownes and Ives). 1987;157:446-7
Bromide
Cord serum bromide concentration: variation and lack of association with pregnancy outcome (Miller et al). 1987;157:826-30
Bruising
Erb's palsy and fetal bruising after external cephalic version: case report (Petrikovsky et al). 1987;157:258
Bulimia
Anorexia nervosa, bulimia, and pregnancy (Stewart et al). 1987;157:1194-8

C

CA-125
Evaluation of serum CA 125 levels in the monitoring of ovarian cancer (Vergote et al). 1987;157:88-92
Serum CA-125 in the evaluation of benign adnexal cysts (Pittaway et al). 1987;157:1426-8
Cadmium
The effect of smoking on placental and fetal zinc status (Kuhnert et al). 1987;157:1241-6
The relationship between cadmium, zinc, and birth weight in pregnant women who smoke (Kuhnert et al). 1987;157:1247-51
Caffeine
Caffeine-cigarette interaction on fetal growth (Beaulac-Baillargeon and Desrosiers). 1987;157:1236-40
The effects of long- and short-term maternal caffeine ingestion on human fetal breathing and body movements in term gestations (McGowan et al). 1987;157:726-9
Calcium
Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes (Ducsay et al). 1987;157:1482-6
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)
Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep (Harake et al). 1987;157:1003-8
Cancer; *see* **Carcinoma**
Capillary hydrostatic pressure
Calculated capillary hydrostatic pressure in normal pregnancy and preeclampsia (Øian and Maltau). 1987;157:102-6
Carbacyclin
Cyclooxygenase inhibition reduces placental transfer: reversal by carbacyclin (Kuhn and Stuart). 1987;157:194-8
Carbohydrates; *see also* **Cerebral glucose**
The "breakfast tolerance test": screening for gestational diabetes with a standardized mixed nutrient meal (Coustan et al). 1987;157:1113-7
Carbon dioxide laser
Carbon dioxide laser laparoscopy performed with a flexible fiber in humans (Baggish et al). 1987;157:1129-33
Carcinoma; *see also* **Malignancy; Neoplasia; Tumor**
Adenosquamous carcinoma of the fallopian tube: a clinicopathologic case report with verification of the diagnosis by immunohistochemical and ultrastructural studies (Moore et al). 1987;157:903-5
The anatomic distribution of cervical adenocarcinoma in situ: implications for treatment (Bertrand et al). 1987;157:21-5
Another look at the second-assessment procedure for ovarian epithelial carcinoma (Gallup et al). 1987;157:590-6
Cigarette smoking and the risk of female reproductive cancer (Stockwell and Lyman). 1987;157:35-40
Decreasing incidence of invasive cervical cancer in young women (Chu and White). 1987;157:1105-7
Endometrial metaplasia associated with endometrial adenocarcinoma (Andersen et al). 1987;157:597-604
Evaluation of serum CA 125 levels in the monitoring of ovarian cancer (Vergote et al). 1987;157:88-92
An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma (Senekjian et al). 1987;157:433-9
Histologic composition of endometrial carcinomas analyzed for steroid receptor content (Soper et al). 1987;157:26-7
Human leukocyte antigen associated with endometrial carcinoma with a new classification of endometrial carcinoma based on its etiology (Kanuma and Igarashi). 1987;157:427-32
Multiple primary gynecologic neoplasms (Rose et al). 1987;157:261-7
Racial differences in survival of women with endometrial cancer (Bain et al). 1987;157:914-23
Cardiac anomaly; *see also* **Anomaly**
Congenital heart block and cardiac anomalies in the absence of maternal connective tissue disease (Shenker et al). 1987;157:248-53
Catecholamines
Acute effects of exercise on plasma catecholamines in sedentary and athletic women with normal and abnormal menses (Chin et al). 1987;157:938-44
Cell volume
γ-Glutamyl transferase and mean cell volume reveal maternal alcohol abuse and fetal alcohol effects (Ylikorkala et al). 1987;157:344-8
Cephalic version
Erb's palsy and fetal bruising after external cephalic version: case report (Petrikovsky et al). 1987;157:258
Cerclage
Management of the diethylstilbestrol-exposed pregnant patient: a prospective study (Ludmir et al). 1987;157:665-9
Cerebellum
Fetal cerebellar growth unaffected by intrauterine growth

retardation: a new parameter for prenatal diagnosis (Reece et al). 1987;157:632-8

Cerebral glucose
Effects of cochlear ablation on local cerebral glucose utilization in fetal sheep (Abrams et al). 1987;157:1438-42
Local cerebral glucose utilization in fetal sheep exposed to noise (Abrams et al). 1987;157:456-60

Cerebral metabolism; *see also* **Cerebral glucose**
Cerebral oxidative metabolism in fetal sheep with multiple-dose ethanol infusion (Richardson et al). 1987;157:1496-502

Cerebral palsy
Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9

Cerebrospinal fluid
Cerebrospinal fluid levels of magnesium in patients with preeclampsia after treatment with intravenous magnesium sulfate: a preliminary report (Thurnau et al). 1987;157:1435-8

Cervical intraepithelial neoplasia
Colposcopic correlates of cervical papillomavirus infection (Follen et al). 1987;157:809-14
Evaluation of the atypical Pap smear (Jones et al). 1987;157:544-9
Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia (Anderson) (Letter); (Baggish) (Reply). 1987;157:1322-3
The results of cryosurgical treatment of cervical intraepithelial neoplasia at one, five, and ten years (Benedet et al). 1987;157:268-73

Cervicitis
Detection of *Chlamydia trachomatis* cervical infection: a comparison of Papanicolaou and immunofluorescent staining with cell culture (Quinn et al). 1987;157:394-9
The use and limitations of endocervical Gram stains and mucopurulent cervicitis as predictors for *Chlamydia trachomatis* in female adolescents (Moscicki et al). 1987;157:65-71

Cervix; *see also* **Cervical intraepithelial neoplasia; Cervicitis**
The anatomic distribution of cervical adenocarcinoma in situ: implications for treatment (Bertrand et al). 1987;157:21-5
Cervical endometriosis: facilitated diagnosis by fine needle aspiration cytologic testing (Veiga-Ferreira et al). 1987;157:849-56
Correlation between chlamydia infection and clinical evaluation, vaginal wet smear, and cervical swab test in female adolescents (Thejls et al). 1987;157:974-6
Decreased elastic fibers and desmosine content in incompetent cervix (Leppert et al). 1987;157:1134-9
Decreasing incidence of invasive cervical cancer in young women (Chu and White). 1987;157:1105-7
An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma (Senekjian et al). 1987;157:433-9
Langerhans' cells in the cervix (Hawthorn and MacLean) (Letter); (McArdle) (Reply). 1987;157:213-5
Management of the diethylstilbestrol-exposed pregnant patient: a prospective study (Ludmir et al). 1987;157:665-9
Sonographic evaluation of the cervix during ovulation induction (Hill et al). 1987;157:1170-4

Cesarean section
Careful surgical technique can reduce infectious morbidity after cesarean section (Lyon and Richardson). 1987;157:557-62
Cost-effective use of antibiotic prophylaxis for cesarean section (Ford et al). 1987;157:506-10
Neonatal seizures after cesarean delivery: higher risk with labor (Spellacy et al). 1987;157:377-9
The pregnant brain dead and the fetus: Must we always try to wrest life from death? (Loewy). 1987;157:1097-101 (Clin. opinion)
Previous cesarean delivery and trial of labor (Fribourg) (Letter); (Finley) (Reply). 1987;157:1014-5
Primary cesarean section and subsequent fertility (LaSala and Berkeley). 1987;157:379-83
Prophylactic antibiotics for cesarean delivery: a simple cost-effective strategy for prevention of postoperative morbidity (Duff). 1987;157:794-8 (Clin. opinion)
A prospective randomized study of the aggressive management of early labor (Cohen et al). 1987;157:1174-7
The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores (Vintzileos et al). 1987;157:627-31
Risk of vaginal delivery after a prior cesarean section (Catanzarite) (Letter). 1987;157:213
Short course of antibiotics for post–cesarean section endometritis (Cabbad et al). 1987;157:908-9
Single-dose versus multiple-dose piperacillin prophylaxis in primary cesarean operation (Gall and Hill). 1987;157:502-6
Vaginal birth after cesarean (Phelan et al). 1987;157:1510-5

Chemicals
Disruption of embryonic and fetal development due to preimplantation chemical insults: a critical review (Iannaccone et al). 1987;157:476-84 (Curr. dev.)

Chemotaxis
Cost-effective use of antibiotic prophylaxis for cesarean section (Ford et al). 1987;157:506-10
Host defense during pregnancy: neutrophil chemotaxis and adherence (Krause et al). 1987;157:274-80

Chlamydia trachomatis
Correlation between chlamydia infection and clinical evaluation, vaginal wet smear, and cervical swab test in female adolescents (Thejls et al). 1987;157:974-6
Detection of *Chlamydia trachomatis* cervical infection: a comparison of Papanicolaou and immunofluorescent staining with cell culture (Quinn et al). 1987;157:394-9
The use and limitations of endocervical Gram stains and mucopurulent cervicitis as predictors for *Chlamydia trachomatis* in female adolescents (Moscicki et al). 1987;157:65-71

Chloroaminobenzoic acid
2-Chloroprocaine for local perineal infiltration (Philipson et al). 1987;157:1275-8

2-Chloroprocaine
2-Chloroprocaine for local perineal infiltration (Philipson et al). 1987;157:1275-8

Cholesteryl palmitate
Cholesteryl palmitate as a predictor of fetal lung maturity (Ludmir et al). 1987;157:84-8

Chorioamnionitis
Leukocyte esterase activity in human amniotic fluid for the rapid detection of chorioamnionitis (Hoskins et al). 1987;157:730-2

Chorion
Progesterone synthesis by human amnion, chorion, and decidua at term (Mitchell et al). 1987;157:349-53

Chorionic gonadotropin; *see* **Gonadotropins**
Chorionic villus
Chorionic villus sampling is associated with normal fetal growth (Williams et al). 1987;157:708-12
Echocardiographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy (Hustin and Schaaps). 1987;157:162-8
Emotional responses of pregnant women to chorionic villi sampling or amniocentesis (Spencer and Cox). 1987;157:1155-60
The immediate effects of chorionic villus sampling on fetal movements (Boogert et al). 1987;157:137-9
Transabdominal chorionic villus sampling: a freehand ultrasound-guided technique (Brambati et al). 1987;157:134-7
Cigarette smoking; *see* **Smoking**
Clinical opinion
Clinical opinion. 1987;157:1-9, 229-35, 789-98, 1093-101
Clomiphene
Absence of clomiphene and/or active metabolites in blood of clomiphene citrate–treated patients during organogenesis: determination by radioreceptor assay (Geier et al) (Letter). 1987;157:1009
Coagulation; *see also* **Blood; Hemorrhage**
Coagulation effects of oral contraception (Bonnar). 1987;157:1042-8
Cocaine
Cocaine use during pregnancy: adverse perinatal outcome (MacGregor et al). 1987;157:686-90
Cochlear ablation
Effects of cochlear ablation on local cerebral glucose utilization in fetal sheep (Abrams et al). 1987;157:1438-42
Colloid osmotic pressure
Mechanisms for reduced colloid osmotic pressure in preeclampsia (Bhatia et al). 1987;157:106-8
Colposcopy
Colposcopic correlates of cervical papillomavirus infection (Follen et al). 1987;157:809-14
Colposcopic evaluation of abnormal Papanicolaou smears in pregnancy (Benedet et al). 1987;157:932-7
Evaluation of the atypical Pap smear (Jones et al). 1987;157:544-9
Congenital anomaly; *see* **Anomaly**; *specific anomaly*
Congenital heart disease
Congenital heart block and cardiac anomalies in the absence of maternal connective tissue disease (Shenker et al). 1987;157:248-53
Fetal echocardiographic screening for congenital heart disease: the importance of the four-chamber view (Copel et al). 1987;157:648-55
Conization
Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia (Anderson) (Letter); (Baggish) (Reply). 1987;157:1322-3
Connective tissue disease
Congenital heart block and cardiac anomalies in the absence of maternal connective tissue disease (Shenker et al). 1987;157:248-53
Consultants
Consultants for the American Journal of Obstetrics and Gynecology. 1987;157:1325-32
Contraception
Coagulation effects of oral contraception (Bonnar). 1987;157:1042-8
Contraception: an overview (Hammerstein). 1987;157:1020-3
Contraceptive use, pelvic inflammatory disease, and fertility problems among American women: 1982 (Aral et al). 1987;157:59-64
Experiences with gossypol as a male pill (Liu et al). 1987;157:1079-81
Hormonal contraception: benefits versus risks (Goldzieher). 1987;157:1023-8
International symposium on contraception. Part 1: oral contraception. 1987;157:1019-63
International symposium on contraception. Part I: oral contraception: introduction (Speroff). 1987;157:1019
International symposium on contraception. Part 2: alternative methods of contraception. 1987;157:1064-92
International symposium on contraception. Part 2: alternative methods of contraception: introduction (Diczfalusy). 1987;157:1064
Metabolic effects of oral contraceptives (Gaspard). 1987;157:1029-41
Natural family planning (Brown et al). 1987;157:1082-9
A new delivery system for contraceptive steroids (Segal). 1987;157:1090-2
New progestogens in oral contraceptives (Runnebaum and Rabe). 1987;157:1059-63
Phasic approach to oral contraceptives (Hale). 1987;157:1052-8
Progestogen potency in oral contraceptive pills (Gillmer). 1987;157:1048-52
Updated pregnancy rates for the Today contraceptive sponge (Edelman and North). 1987;157:1164-5
Venous thrombosis in patients with cardiac pacemakers and oral contraceptive use (Rao) (Letter). 1987;157:516-7
Contraction
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)
A prospective evaluation of nipple stimulation techniques for contraction stress testing (Keegan et al). 1987;157:121-5
Copper
Serum copper level in gynecologic malignancies (Margalioth et al). 1987;157:93-6
Cord; *see* **Umbilical cord**
Cord blood
Cord sampling for the evaluation of oxygenation and acid-base balance in human growth-retarded fetuses (Pardi et al). 1987;157:1221-8
Cord serum bromide concentration: variation and lack of association with pregnancy outcome (Miller et al). 1987;157:826-30
Enhancement of maternal and neonatal natural killer cell activity with interleukin-2 (Baker et al). 1987;157:780-1
The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores (Vintzileos et al). 1987;157:627-31
Riboflavin concentration in maternal and cord blood in human pregnancy (Kirshenbaum et al). 1987;157:748-52
Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9
Umbilical cord blood pH and Apgar score as an index of neonatal health (Josten et al). 1987;157:843-8
Cordotomy
The effect of severing the spinal cord of fetal lambs on length of gestation (Nowak et al). 1987;157:464-7
Coronary heart disease
Menstrual and reproductive factors and the risk of myo-

cardial infarction in women under 55 years of age (La Vecchia et al). 1987;157:1108-12

Corrections

Echocardiographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy (Hustin and Schaaps) (1987;157:162-8). 1987; 157:1291

Effects of maternal anemia on cardiac output, systemic oxygen consumption, and regional blood flow in pregnant sheep (Edelstone et al) (1987;156:740-8). 1987;157:1291

Inhibition of prolactin secretion by antiserum to the α-and β-subunits of gonadotropin (Blackwell and Garrison) (1987;156:863-8). 1987;157:1520

Predictive value of early pregnancy glycohemoglobin in the insulin-treated diabetic patient (Key et al) (1987;156:1096-100). 1987;157:1460

Proportional weight gain and complications of pregnancy, labor, and delivery in healthy women of normal prepregnant stature (Shepard et al) (1986;155:947-54). 1987;157:217

The role of platelet-activating factor in human fetal lung maturation (Hoffman et al) (1986;155:70-5). 1987; 157:179

Correspondence

Letters. 1987;157:212-7, 513-8, 782-6, 1009-16, 1316-23

Corticotropin-releasing hormone

Pituitary responses to synthetic corticotropin-releasing hormone: absence of modulatory effects by estrogen and progestin (Liu et al). 1987;157:1387-91

Cortisol

The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation (Towell et al). 1987;157:1550-7

Creatinine phosphokinase

Indicators of perinatal asphyxia (Hollander et al). 1987;157:839-42

Cryosurgery

The results of cryosurgical treatment of cervical intraepithelial neoplasia at one, five, and ten years (Benedet et al). 1987;157:268-73

Curettage

The value of curettage in the diagnosis of ectopic pregnancy (Ollendorff et al). 1987;157:71-2

Current development

Current development. 1987;157:180-4, 476-84, 1303-15

Cyclic adenosine monophosphate

Changes in cyclic adenosine monophosphate–phosphodiesterase activity in nonpregnant and pregnant human myometrium (Kofinas et al). 1987;157:733-8

A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)

Cyclooxygenase

Cyclooxygenase inhibition reduces placental transfer: reversal by carbacyclin (Kuhn and Stuart). 1987;157:194-8

Cystic adenomatoid malformation

Successful fetal therapy for cystic adenomatoid malformation associated with second-trimester hydrops (Clark et al). 1987;157:294-5

Cysts

Breast galactography in the treatment of infected cysts (Tihansky and DiBlasio). 1987;157:1278-80

Serum CA-125 in the evaluation of benign adnexal cysts (Pittaway et al). 1987;157:1426-8

Theca-lutein ovarian cysts associated with homozygous α-thalassemia (Miller et al). 1987;157:912-4

Cytogenetics

Hydatidiform mole: cytogenetically unusual cases and their implications for the present classification (Vejerslev et al). 1987;157:180-4 (Curr. dev.)

D

Danazol

The morphologic effect of short-term medical therapy of endometriosis (Brosens et al). 1987;157:1215-21

1-Deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin

Evaluation of 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: a new tocolytic agent (Hahn et al). 1987;157:977-82

Death; *see also* **Mortality**

Factors affecting psychological adjustment to a fetal death (Graham et al). 1987;157:254-7

Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths (Manning et al). 1987;157:880-4

Fetal death: diagnosis and management (Pitkin). 1987;157:583-9

Decidua

Progesterone synthesis by human amnion, chorion, and decidua at term (Mitchell et al). 1987;157:349-53

Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua (Challis and Vaughan). 1987;157:1474-81

Delivery; *see also* **Preterm labor and delivery**

Preeclampsia, delivery, and the hemostatic system (Saleh et al). 1987;157:331-6

Risk of vaginal delivery after a prior cesarean section (Catanzarite) (Letter). 1987;157:213

Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant (Druzin et al). 1987;157:1503-10

Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9

Deoxyribonucleic acid

Partial hydatidiform moles: deoxyribonucleic acid content and course (David et al). 1987;157:969-73

Desmosine

Decreased elastic fibers and desmosine content in incompetent cervix (Leppert et al). 1987;157:1134-9

Diabetes mellitus

The "breakfast tolerance test": screening for gestational diabetes with a standardized mixed nutrient meal (Coustan et al). 1987;157:1113-7

Early third-trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term (Bochner et al). 1987;157:703-8

Gestational diabetes: insulin requirements in pregnancy (Langer et al). 1987;157:669-75

Gestational diabetes: maternal response to diet and insulin therapy as reflected by glycosylated hemoglobin concentration (Grandis et al). 1987;157:1118-21

A hypothesis concerning the general basis of organogenetic congenital anomalies (Naftolin et al). 1987;157:1-4 (Clin. opinion)

Necrotizing fasciitis of the vulva (Roberts). 1987;157:568-71

Postprandial walking exercise in pregnant insulin-depen-

dent (type 1) diabetic women: reduction of plasma lipid levels but absence of a significant effect on glycemic control (Hollingsworth and Moore). 1987;157:1359-63
The reliability and clinical use of a rapid phosphatidylglycerol assay in normal and diabetic pregnancies (Saad et al). 1987;157:1516-20
Restriction fragment length polymorphisms of the insulin gene hypervariable region in gestational onset diabetes mellitus (Ober et al). 1987;157:1364-8
The significance of one abnormal glucose tolerance test value on adverse outcome in pregnancy (Langer et al). 1987;157:758-63
Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short-and long-term metabolic control in gestational diabetes (Brustman et al). 1987;157:699-703

Diagnostic imaging
Diagnostic imaging of gynecologic tumors with the monoclonal antibody 791T/36 (Powell et al). 1987;157:28-34

Diet
Gestational diabetes: maternal response to diet and insulin therapy as reflected by glycosylated hemoglobin concentration (Grandis et al). 1987;157:1118-21

Diethylstilbestrol
Management of the diethylstilbestrol-exposed pregnant patient: a prospective study (Ludmir et al). 1987;157:665-9

Digoxin
Digoxin-like immunoreactive substance in fetuses with and without cardiac pathology (Weiner et al). 1987;157:368-71
Digoxin-like immunoreactive substance in pregnancy (Gonzalez et al). 1987;157:660-4
Insufficient transplacental digoxin transfer in severe hydrops fetalis (Younis and Granat). 1987;157:1268-9

Diphenhydramine
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91

Dipyridamole
Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole (Wallenburg and Rotmans). 1987;157:1230-5

Doppler study
Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow (Reed et al). 1987;157:774-9
A classification of hypertension in pregnancy based on Doppler velocimetry (Ducey et al). 1987;157:680-5
Renal arterial blood flow velocity waveforms in pregnant women (Hata et al). 1987;157:1269-71
Umbilical artery flow velocity waveforms and the placental vascular bed (McCowan et al). 1987;157:900-2
Uterine artery Doppler velocimetry: the significance of divergent systolic/diastolic ratios (Shulman et al). 1987;157:1539-42
Uterine and umbilical artery velocimetry during normal labor (Fleischer et al). 1987;157:40-3

Down syndrome
Soft tissue nuchal fold in the second-trimester fetus: standards for normal measurements compared with those in Down syndrome (Benacerraf and Frigoletto). 1987;157:1146-9
A sonographic screening method for Down syndrome (Lockwood et al). 1987;157:803-8

Drug abuse
Cocaine use during pregnancy: adverse perinatal outcome (MacGregor et al). 1987;157:686-90
γ-Glutamyl transferase and mean cell volume reveal maternal alcohol abuse and fetal alcohol effects (Ylikorkala et al). 1987;157:344-8
Mycotic aneurysms in pregnancy (Boike et al). 1987;157:340-1

Drugs
Disruption of embryonic and fetal development due to preimplantation chemical insults: a critical review (Iannaccone et al). 1987;157:476-84 (Curr. dev.)
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91
Drug prescribing for thromboembolic disease during pregnancy (Greenspoon and Montoro) (Letter). 1987;157:784-5
Prescription drug use before and during pregnancy in a Medicaid population (Piper et al). 1987;157:148-56

DRw8 antigen
Recurrent spontaneous abortion and human leukocyte antigen DRw8 (Takamizawa et al) (Letter). 1987;157:514-5

Dysmenorrhea
Simultaneous objective and subjective evaluation of meclofenamate sodium in the treatment of primary dysmenorrhea (Smith and Powell). 1987;157:611-8

Dystocia
Early third-trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term (Bochner et al). 1987;157:703-8
A prospective randomized study of the aggressive management of early labor (Cohen et al). 1987;157:1174-7

E

Eating disorders
Anorexia nervosa, bulimia, and pregnancy (Stewart et al). 1987;157:1194-8

Echocardiography
Echocardiographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy (Hustin and Schaaps). 1987;157:162-8
Fetal echocardiographic screening for congenital heart disease: the importance of the four-chamber view (Copel et al). 1987;157:648-55

Eclampsia; *see also* **Preeclampsia**
Blood pressure in the midtrimester and future eclampsia (Chesley and Sibai). 1987;157:1258-61

Ectopic pregnancy
Abdominal pregnancy: magnetic resonance identification with ultrasonographic follow-up of placental involution (Spanta et al). 1987;157:887-9
Conservative surgical management of isthmic ectopic pregnancies (Smith et al). 1987;157:604-10
First-trimester serum levels of the β-subunit of human chorionic gonadotropin in a tubal molar pregnancy (Chase et al). 1987;157:910
Hysterosalpingographic evaluation of tubal patency after ectopic pregnancy (Mitchell et al). 1987;157:618-22
The value of curettage in the diagnosis of ectopic pregnancy (Ollendorff et al). 1987;157:71-2

Effusion
Pleural effusions and pulmonary hypoplasia (Castillo et al). 1987;157:1252-5

Elastic fiber
Decreased elastic fibers and desmosine content in incompetent cervix (Leppert et al). 1987;157:1134-9
Electrolytes
The nonpredictive value of fetal urinary electrolytes: preliminary report of outcomes and correlation with pathologic diagnosis (Wilkins et al). 1987;157:694-8
Electromyography
Characteristics of the electromyogram recorded from the mesometrium of the pregnant ewe from 106 days' gestation to delivery: similarities with and differences from the electromyogram obtained from the myometrium (Figueroa et al). 1987;157:991-8
Electron microscopy
Adenosquamous carcinoma of the fallopian tube: a clinicopathologic case report with verification of the diagnosis by immunohistochemical and ultrastructural studies (Moore et al). 1987;157:903-5
Electronic fetal monitoring; *see* **Fetal monitoring**
Electrophoresis
Seminal plasma proteins of fertile and infertile men analyzed by two-dimensional electrophoresis (Ayyagari et al). 1987;157:1528-33
Embolization
Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation (Trudinger et al). 1987;157:1443-8
Embryo
Disruption of embryonic and fetal development due to preimplantation chemical insults: a critical review (Iannaccone et al). 1987;157:476-84 (Curr. dev.)
Human embryo viability related to freezing and thawing procedures (Testart et al). 1987;157:168-71
Hypoglycemia: How little is too much for the embryo? (Sadler and Hunter). 1987;157:190-3
Emotions; *see also* **Psychological adjustment**
Emotional responses of pregnant women to chorionic villi sampling or amniocentesis (Spencer and Cox). 1987;157:1155-60
Endometrial carcinoma
Cigarette smoking and the risk of female reproductive cancer (Stockwell and Lyman). 1987;157:35-40
Endometrial adenocarcinoma estrogen receptor content: association of clinicopathologic features with immunohistochemical analysis compared with standard biochemical methods (Mutch et al). 1987;157:924-31
Endometrial metaplasia associated with endometrial adenocarcinoma (Andersen et al). 1987;157:597-604
Histologic composition of endometrial carcinomas analyzed for steroid receptor content (Soper et al). 1987;157:26-7
Human leukocyte antigen associated with endometrial carcinoma with a new classification of endometrial carcinoma based on its etiology (Kanuma and Igarashi). 1987;157:427-32
Racial differences in survival of women with endometrial cancer (Bain et al). 1987;157:914-23
Endometriosis
Cervical endometriosis: facilitated diagnosis by fine needle aspiration cytologic testing (Veiga-Ferreira et al). 1987;157:849-56
Effect of pregnancy on surgically induced endometriosis in cynomolgus monkeys (Schenken et al). 1987;157:1392-6
Local peritoneal factors: their role in infertility associated with endometriosis (Awadalla et al). 1987;157:1207-14
The morphologic effect of short-term medical therapy of endometriosis (Brosens et al). 1987;157:1215-21
Endometritis
Prophylactic antibiotics for cesarean delivery: a simple cost-effective strategy for prevention of postoperative morbidity (Duff). 1987;157:794-8 (Clin. opinion)
Short course of antibiotics for post–cesarean section endometritis (Cabbad et al). 1987;157:908-9
Endometrium
Characteristics of the electromyogram recorded from the mesometrium of the pregnant ewe from 106 days' gestation to delivery: similarities with and differences from the electromyogram obtained from the myometrium (Figueroa et al). 1987;157:991-8
The effect of insulin on aromatase activity in isolated human endometrial glands and stroma (Randolph et al). 1987;157:1534-9
Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs (Elger et al). 1987;157:1065-74
Suppression of lymphocyte reactivity in vitro by supernatants of explants of human endometrium (Wang et al). 1987;157:956-63
β-Endorphin
Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: changes during the menstrual cycle (Petraglia et al). 1987;157:142-6
Endotoxin
Infection and labor: the detection of endotoxin in amniotic fluid (Romero et al). 1987;157:815-9
Energy metabolism
Placental glycolysis and energy metabolism in preeclampsia (Bloxam et al). 1987;157:97-101
Enzyme immunoassay
Natural family planning (Brown et al). 1987;157:1082-9
Enzymes
Increased tubular enzyme excretion in preeclampsia (Goren et al). 1987;157:906-8
Epithelial carcinoma
Another look at the second-assessment procedure for ovarian epithelial carcinoma (Gallup et al). 1987;157:590-6
Erb's palsy
Erb's palsy and fetal bruising after external cephalic version: case report (Petrikovsky et al). 1987;157:258
Errata; *see* **Corrections**
Erythroblastosis fetalis
Measurement of human fetoplacental blood volume in erythroblastosis fetalis (Nicolaides et al). 1987;157:50-3
Estradiol
Modulating actions of estradiol on gonadotropin-releasing hormone–stimulated prolactin secretion in postmenopausal individuals (Christiansen et al). 1987;157:320-5
Estriol
Human chorionic gonadotropin, estriol, and testosterone changes in two pregnancies with hyperreactio luteinalis (Muechler et al). 1987;157:1126-8
Estrogens
Age-related changes in the female hormonal environment during reproductive life (Musey et al). 1987;157:312-7
Coagulation effects of oral contraception (Bonnar). 1987;157:1042-8
Continuous estrogen/progestin therapy in menopause (Prough et al). 1987;157:1449-53
Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys (Billiar et al). 1987;157:1297-302
Endometrial adenocarcinoma estrogen receptor content: association of clinicopathologic features with immunohis-

tochemical analysis compared with standard biochemical methods (Mutch et al). 1987;157:924-31
Estrogen, progesterone, prolactin, prostaglandin E_2, prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ gradients across the uterus in women in labor and not in labor (Davidson et al). 1987;157:54-8
Evidence that estrogen may be a key factor in hyperprolactinemic anovulation: a case report (Sakamoto et al). 1987;157:318-9
Histologic composition of endometrial carcinomas analyzed for steroid receptor content (Soper et al). 1987;157:26-7
Menstrual and reproductive factors and the risk of myocardial infarction in women under 55 years of age (La Vecchia et al). 1987;157:1108-12
Natural family planning (Brown et al). 1987;157:1082-9
Pituitary responses to synthetic corticotropin-releasing hormone: absence of modulatory effects by estrogen and progestin (Liu et al). 1987;157:1387-91

Ethanol; *see also* **Alcohol**
Cerebral oxidative metabolism in fetal sheep with multiple-dose ethanol infusion (Richardson et al). 1987;157:1496-502
Disposition of acute, multiple-dose ethanol in the near-term pregnant ewe (Brien et al). 1987;157:204-11

Ethics
When does a statistical fact become an ethical imperative? (Silver and Minogue). 1987;157:229-33 (Clin. opinion)

Exercise
Acute effects of exercise on plasma catecholamines in sedentary and athletic women with normal and abnormal menses (Chin et al). 1987;157:938-44
Effects of aerobic and strength conditioning on pregnancy outcomes (Hall and Kaufmann). 1987;157:1199-203

F

Facial anomaly
The detection of facial anomalies with ultrasound (Koontz) (Letter); (Pilu) (Reply). 1987;157:1316

Factor XI deficiency
Factor XI deficiency presenting as hemarthrosis during pregnancy (Musclow et al). 1987;157:178-9

Fallopian tube
Adenosquamous carcinoma of the fallopian tube: a clinicopathologic case report with verification of the diagnosis by immunohistochemical and ultrastructural studies (Moore et al). 1987;157:903-5

False results
Definitions of sensitivity, specificity, false negative results, and false positive results (Kolins) (Letter). 1987;157:517-8
Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths (Manning et al). 1987;157:880-4

Femininity
Femininity—a problem(?) (Eichner) (Letter). 1987;157:517

Femur
Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements (Vintzileos et al). 1987;157:410-4

Fertilization; *see also* **Infertility**
Sonographic evaluation of the cervix during ovulation induction (Hill et al). 1987;157:1170-4
Transurethral ultrasound-guided oocyte retrieval for in vitro fertilization (Padilla et al). 1987;157:622-6

Fetal alcohol syndrome
γ-Glutamyl transferase and mean cell volume reveal maternal alcohol abuse and fetal alcohol effects (Ylikorkala et al). 1987;157:344-8
Stereotypic behavioral response of rat fetuses to acute hypoxia is altered by maternal alcohol consumption (Smotherman and Robinson). 1987;157:982-6

Fetal biophysical profile
Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths (Manning et al). 1987;157:880-4
The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores (Vintzileos et al). 1987;157:627-31
The use of fetal biophysical profile improves pregnancy outcome in premature rupture of the membranes (Vintzileos et al). 1987;157:236-40

Fetal blood
In vitro training model for diagnostic and therapeutic fetal intravascular needle puncture (Timor-Tritsch and Yeh). 1987;157:858-9
Intrapartum use of fetal scalp hematocrit in the diagnosis of profound fetal anemia caused by fetomaternal hemorrhage (Herman et al). 1987;157:1182-3
Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep (Harake et al). 1987;157:1003-8
Percutaneous ultrasound-guided fetal blood sampling in the management of nonimmune hydrops fetalis (Hsieh et al). 1987;157:44-9
Scalp heat flux and its relationship to scalp blood pH of the fetus (Rudelstorfer et al). 1987;157:372-7
Validity of intrapartum diagnosis of fetal thrombocytopenia (Christiaens and Helmerhorst). 1987;157:864-5

Fetal death
Factors affecting psychological adjustment to a fetal death (Graham et al). 1987;157:254-7
Fetal death: diagnosis and management (Pitkin). 1987;157:583-9

Fetal distress
Antepartum predictors of fetal distress in postterm pregnancy (Bochner et al). 1987;157:353-8
Fetal monitoring during emergency obstetric transport (Elliott and Trujillo). 1987;157:245-7
Obstetric outcome of patients with a previous episode of spurious labor (Arulkumaran et al). 1987;157:17-20
A study on intrauterine fetal resuscitation with terbutaline (Patriarco et al). 1987;157:384-7

Fetal growth; *see also* **Intrauterine growth retardation**
Disruption of embryonic and fetal development due to preimplantation chemical insults: a critical review (Iannaccone et al). 1987;157:476-84 (Curr. dev.)
Soft tissue nuchal fold in the second-trimester fetus: standards for normal measurements compared with those in Down syndrome (Benacerraf and Frigoletto). 1987;157:1146-9
Sonographic amniotic fluid measurement and fetal growth retardation: a reappraisal (Patterson et al). 1987;157:1406-10
Sonographic features of the triploid fetus (Lockwood et al). 1987;157:285-7

Fetal hydrops; *see* **Hydrops fetalis**

Fetal lung maturity
Cholesteryl palmitate as a predictor of fetal lung maturity (Ludmir et al). 1987;157:84-8

Fetal pulmonary hypoplasia with hydrothorax (Murayama et al). 1987;157:119-20
Pleural effusions and pulmonary hypoplasia (Castillo et al). 1987;157:1252-5
Transplacental stimulation of functional and morphologic fetal rabbit lung maturation: effect of thyrotropin-releasing hormone (Devaskar et al). 1987;157:460-4
Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia (Johnson et al). 1987;157:764-9

Fetal membranes
Amniotic fluid trypsin and premature rupture of membranes (McGregor et al) (Letter); (Kanayama) (Reply). 1987;157:1316-9
The effects of membrane permeability and binding by human serum proteins on steroid influx into the rabbit uterus (Steingold et al). 1987;157:1543-9
In vitro conversion of pregnenolone to progesterone in human term placenta and fetal membranes before and after onset of labor (Khan-Dawood). 1987;157:1333-7
Increased boy-to-girl ratio in women with preterm labor beginning with contractions (Seki and Kato) (Letter). 1987;157:215-6
Management of preterm premature rupture of membranes: lack of a national consensus (Capeless and Mead). 1987;157:11-2
The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor (Besinger et al). 1987;157:753-7
A randomized trial of ritodrine tocolysis versus expectant management in patients with premature rupture of membranes at 25 to 30 weeks of gestation (Garite et al). 1987;157:388-93
Reduction of group B streptococcal maternal and neonatal infections in preterm pregnancies with premature rupture of membranes through a rapid identification test (Morales and Lim). 1987;157:13-6
Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua (Challis and Vaughan). 1987;157:1474-81
Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia (Johnson et al). 1987;157:764-9
The use of fetal biophysical profile improves pregnancy outcome in premature rupture of the membranes (Vintzileos et al). 1987;157:236-40

Fetal monitoring; *see also* **Acoustic stimulation**
Antepartum fetal surveillance in the patient with decreased fetal movement (Ahn et al). 1987;157:860-4
Breast stimulation test and oxytocin challenge test in fetal surveillance: a prospective randomized study (Lipitz et al). 1987;157:1178-81
Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow (Reed et al). 1987;157:774-9
The effects of maternal ambulation on the nonstress test (Devoe et al). 1987;157:240-4
Fetal heart rate monitoring (Dawes and Redman) (Letter); (Fukushima et al) (Reply). 1987;157:513-4
Fetal heart rate response to vibratory acoustic stimulation predicts fetal pH in labor (Edersheim et al). 1987;157:1557-60
Fetal monitoring during emergency obstetric transport (Elliott and Trujillo). 1987;157:245-7
Heart rate variation and movement incidence in growth-retarded fetuses: the significance of antenatal late heart rate decelerations (Bekedam et al). 1987;157:126-33
Intrapartum electronic fetal monitoring efficacy (Fribourg) (Letter); (Thacker) (Reply). 1987;157:1013-4
Intrapartum vibratory acoustic stimulation of the human fetus during episodes of decreased heart rate variability (Divon et al). 1987;157:1355-8
Paper speed in electronic fetal heart rate monitoring (Floyd) (Letter); (Goodlin) (Reply). 1987;157:212
Patterns of human fetal heart rate accelerations from 26 weeks to term (Gagnon et al). 1987;157:743-8
Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant (Druzin et al). 1987;157:1503-10
Umbilical blood waveforms in fetal surveillance of twins (Saldana et al). 1987;157:712-5

Fetal movement
Antepartum fetal surveillance in the patient with decreased fetal movement (Ahn et al). 1987;157:860-4
The effects of long-and short-term maternal caffeine ingestion on human fetal breathing and body movements in term gestations (McGowan et al). 1987;157:726-9
The immediate effects of chorionic villus sampling on fetal movements (Boogert et al). 1987;157:137-9
The length of the human umbilical cord in twin pregnancies (Soernes and Bakke). 1987;157:1229-30
The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor (Besinger et al). 1987;157:753-7
The use of fetal neuromuscular blockade during intrauterine procedures (Moise et al). 1987;157:874-9

Fetomaternal hemorrhage
Fetal death: diagnosis and management (Pitkin). 1987;157:583-9
Intrapartum use of fetal scalp hematocrit in the diagnosis of profound fetal anemia caused by fetomaternal hemorrhage (Herman et al). 1987;157:1182-3
Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage (Cardwell). 1987;157:358-9

Fetoplacental blood
Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons (Howard et al). 1987;157:1261-6
Measurement of human fetoplacental blood volume in erythroblastosis fetalis (Nicolaides et al). 1987;157:50-3

α-Fetoprotein
Amniotic fluid α-fetoprotein levels and pregnancy outcome (Brumfield et al). 1987;157:822-5
Elevated maternal serum α-fetoprotein levels and oligohydramnios: poor prognosis for pregnancy outcome (Dyer et al). 1987;157:336-9
Maternal serum α-fetoprotein screening: effects of weight adjustment (Kazazian et al) (Letter); (Macri et al) (Reply). 1987;157:782-4
Maternal serum α-fetoprotein screening. III. Pitfalls in evaluating black gravid women (Macri et al). 1987;157:820-2
Outcome in patients with unusually high maternal serum α-fetoprotein levels (Nelson et al). 1987;157:572-6

Fetus
Acute atherosis in preeclampsia: maternal determinants and fetal outcome in the presence of the lesion (Khong et al). 1987;157:360-3
Acute oligohydramnios in postdate pregnancy (Clement et al). 1987;157:884-6

The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly (Carr and Parker). 1987;157:1351-5
Breakage of a fetal scalp blade with retention of fragments in the infant's scalp (Nieburg and Gross). 1987;157:441-2
Cerebral oxidative metabolism in fetal sheep with multiple-dose ethanol infusion (Richardson et al). 1987;157:1496-502
Cord sampling for the evaluation of oxygenation and acid-base balance in human growth-retarded fetuses (Pardi et al). 1987;157:1221-8
Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow (Reed et al). 1987;157:774-9
Digoxin-like immunoreactive substance in fetuses with and without cardiac pathology (Weiner et al). 1987;157:368-71
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91
The effect of severing the spinal cord of fetal lambs on length of gestation (Nowak et al). 1987;157:464-7
The effect of smoking on placental and fetal zinc status (Kuhnert et al). 1987;157:1241-6
Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes (Ducsay et al). 1987;157:1482-6
Effects of cochlear ablation on local cerebral glucose utilization in fetal sheep (Abrams et al). 1987;157:1438-42
The effects of long-and short-term maternal caffeine ingestion on human fetal breathing and body movements in term gestations (McGowan et al). 1987;157:726-9
Effects of maternal glucose infusion on fetal acid-base status in human pregnancy (Philipson et al). 1987;157:866-73
Effects of norepinephrine on lung fluid flow rate in the chronically catheterized fetal lamb (Higuchi et al). 1987;157:986-90
Erb's palsy and fetal bruising after external cephalic version: case report (Petrikovsky et al). 1987;157:258
Fetal echocardiographic screening for congenital heart disease: the importance of the four-chamber view (Copel et al). 1987;157:648-55
Fetal umbilical artery flow velocimetry in postdate pregnancies (Guidetti et al). 1987;157:1521-3
Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements (Vintzileos et al). 1987;157:410-4
Fetal-induced trauma as a cause of antepartum hemorrhage (Eden). 1987;157:830-1
Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes (Shinozuka et al). 1987;157:1140-5
Human fetal breathing response to intravenous glucose is directly related to gestational age (Harper et al). 1987;157:1403-5
Increased boy-to-girl ratio in women with preterm labor beginning with contractions (Seki and Kato) (Letter). 1987;157:215-6
Insufficient transplacental digoxin transfer in severe hydrops fetalis (Younis and Granat). 1987;157:1268-9
Local cerebral glucose utilization in fetal sheep exposed to noise (Abrams et al). 1987;157:456-60
Metabolism of lipoproteins by human fetal hepatocytes (Carr). 1987;157:1338-44
The nonpredictive value of fetal urinary electrolytes: preliminary report of outcomes and correlation with pathologic diagnosis (Wilkins et al). 1987;157:694-8
Pemphigus in pregnancy: a reevaluation of fetal risk (Metzker and Merlob) (Letter). 1987;157:1012-3
Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops (Socol et al). 1987;157:1369-75
Pleural effusions and pulmonary hypoplasia (Castillo et al). 1987;157:1252-5
Pregnancy outcome in medically complicated and uncomplicated patients aged 40 years or older (Lehmann and Chism). 1987;157:738-42
The pregnant brain dead and the fetus: Must we always try to wrest life from death? (Loewy). 1987;157:1097-101 (Clin. opinion)
The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor (Besinger et al). 1987;157:753-7
The relationship between cadmium, zinc, and birth weight in pregnant women who smoke (Kuhnert et al). 1987;157:1247-51
Spontaneous fetal seizures in utero (Maouris) (Letter). 1987;157:1009-10
A study on intrauterine fetal resuscitation with terbutaline (Patriarco et al). 1987;157:384-7
Successful fetal therapy for cystic adenomatoid malformation associated with second-trimester hydrops (Clark et al). 1987;157:294-5
Ultrasonic prediction of birth weight in preterm fetuses: Which formula is best? (Pielet et al). 1987;157:1411-4
Ultrasound evaluation of tachycardia-induced hydrops in the fetal lamb (Nimrod et al). 1987;157:655-9
The use of fetal neuromuscular blockade during intrauterine procedures (Moise et al). 1987;157:874-9
Vasopressin-induced bradycardia in fetal and adult sheep is not dependent on an increase in blood pressure (Harper and Rose). 1987;157:448-53

Fever

Careful surgical technique can reduce infectious morbidity after cesarean section (Lyon and Richardson). 1987;157:557-62
The normal parturient's admission temperature (Acker et al). 1987;157:308-11

Fibroblast

The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly (Carr and Parker). 1987;157:1351-5

Fibronectin

Mechanisms for reduced colloid osmotic pressure in preeclampsia (Bhatia et al). 1987;157:106-8
Preeclampsia, delivery, and the hemostatic system (Saleh et al). 1987;157:331-6

Fine needle aspiration

Cervical endometriosis: facilitated diagnosis by fine needle aspiration cytologic testing (Veiga-Ferreira et al). 1987;157:849-56

Follicle-stimulating hormone

Follicle-stimulating hormone is the main determinant of follicular recruitment and development in ovulation induction with human menopausal gonadotropin (Vermesh and Kletzky). 1987;157:1397-402

Follicular fluid

Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: changes during the menstrual cycle (Petraglia et al). 1987;157:142-6

Freezing of embryo
Human embryo viability related to freezing and thawing procedures (Testart et al). 1987;157:168-71
Fungal infection
Mycotic aneurysms in pregnancy (Boike et al). 1987;157:340-1

G

Galactography
Breast galactography in the treatment of infected cysts (Tihansky and DiBlasio). 1987;157:1278-80
GBS-Test
A screening test (GBS-Test) for urogenital carriage of group B streptococci (Christensen and Christensen). 1987;157:341-2
Genetics
Genetic heterogeneity of prematurity and intrauterine growth retardation: clues from the Old Order Amish (Khoury and Cohen). 1987;157:400-10
Restriction fragment length polymorphisms of the insulin gene hypervariable region in gestational onset diabetes mellitus (Ober et al). 1987;157:1364-8
Gestation length
The effect of severing the spinal cord of fetal lambs on length of gestation (Nowak et al). 1987;157:464-7
Gestational age
Human fetal breathing response to intravenous glucose is directly related to gestational age (Harper et al). 1987;157:1403-5
Patterns of human fetal heart rate accelerations from 26 weeks to term (Gagnon et al). 1987;157:743-8
Gestational diabetes mellitus; *see* **Diabetes mellitus**
Gestational trophoblastic disease
Treatment of nonmetastatic gestational trophoblastic disease with oral methotrexate (Barter et al). 1987;157:1166-8
Gestodene
New progestogens in oral contraceptives (Runnebaum and Rabe). 1987;157:1059-63
Gestrinone
The morphologic effect of short-term medical therapy of endometriosis (Brosens et al). 1987;157:1215-21
Glucocorticoids
Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8
Glucose; *see also* **Diabetes mellitus**
The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation (Towell et al). 1987;157:1550-7
Effects of cochlear ablation on local cerebral glucose utilization in fetal sheep (Abrams et al). 1987;157:1438-42
Effects of maternal glucose infusion on fetal acid-base status in human pregnancy (Philipson et al). 1987;157:866-73
Human fetal breathing response to intravenous glucose is directly related to gestational age (Harper et al). 1987;157:1403-5
Local cerebral glucose utilization in fetal sheep exposed to noise (Abrams et al). 1987;157:456-60
Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short-and long-term metabolic control in gestational diabetes (Brustman et al). 1987;157:699-703
Glucose tolerance
The "breakfast tolerance test": screening for gestational diabetes with a standardized mixed nutrient meal (Coustan et al). 1987;157:1113-7
Chronic oral terbutaline tocolytic therapy is associated with maternal glucose intolerance (Main et al). 1987;157:644-7
Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys (Billiar et al). 1987;157:1297-302
Influence of the menstrual cycle on glucose tolerance and insulin secretion (Bonora et al). 1987;157:140-1
Metabolic effects of oral contraceptives (Gaspard). 1987;157:1029-41
The significance of one abnormal glucose tolerance test value on adverse outcome in pregnancy (Langer et al). 1987;157:758-63
Glucuronide
Natural family planning (Brown et al). 1987;157:1082-9
γ-Glutamyl transferase
γ-Glutamyl transferase and mean cell volume reveal maternal alcohol abuse and fetal alcohol effects (Ylikorkala et al). 1987;157:344-8
Glycolysis
Placental glycolysis and energy metabolism in preeclampsia (Bloxam et al). 1987;157:97-101
Glycosylation
Gestational diabetes: maternal response to diet and insulin therapy as reflected by glycosylated hemoglobin concentration (Grandis et al). 1987;157:1118-21
Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short-and long-term metabolic control in gestational diabetes (Brustman et al). 1987;157:699-703
Gonadotropins
Age-related changes in the female hormonal environment during reproductive life (Musey et al). 1987;157:312-7
First-trimester serum levels of the β-subunit of human chorionic gonadotropin in a tubal molar pregnancy (Chase et al). 1987;157:910
Follicle-stimulating hormone is the main determinant of follicular recruitment and development in ovulation induction with human menopausal gonadotropin (Vermesh and Kletzky). 1987;157:1397-402
Human chorionic gonadotropin, estriol, and testosterone changes in two pregnancies with hyperreactio luteinalis (Muechler et al). 1987;157:1126-8
Insulin resistance and abnormal ovarian responses to human chorionic gonadotropin in chronically anovulatory women (Kustin et al). 1987;157:1468-73
Modulating actions of estradiol on gonadotropin-releasing hormone–stimulated prolactin secretion in postmenopausal individuals (Christiansen et al). 1987;157:320-5
Observations on the log human chorionic gonadotropin–time relationship in early pregnancy and its practical implications (Kadar and Romero). 1987;157:73-8
Recent developments in immunocontraception (Talwar and Gaur). 1987;157:1075-8
Gossypol
Experiences with gossypol as a male pill (Liu et al). 1987;157:1079-81
Gram stains
The use and limitations of endocervical Gram stains and mucopurulent cervicitis as predictors for *Chlamydia tra-*

chomatis in female adolescents (Moscicki et al). 1987;157:65-71

Grief

Evaluation of a perinatal grief support team (Lake et al). 1987;157:1203-6

Growth; *see* **Fetal growth; Intrauterine growth retardation**

Growth hormone

Growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels compared in premature, small, average birth weight, and large infants (Samaan et al). 1987;157:1524-8

In vivo and in vitro modulation of growth hormone and prolactin of a mixed somatotropic-lactotropic pituitary microadenoma (Muechler et al). 1987;157:453-5

Gynecology

Mechanical intestinal obstruction in patients with gynecologic disease: a review of 368 patients (Krebs and Goplerud). 1987;157:577-83

Urogynecology: comments and caveats (Thiede and Saini). 1987;157:563-8

H

Haemophilus influenzae

Haemophilus influenzae infection and midtrimester abortion (Amstey and Ogden) (Letter). 1987;157:518

Handedness

The umbilical cord twist: origin, direction, and relevance (Lacro et al). 1987;157:833-8

Haptoglobin

Haptoglobin helps diagnose the HELLP syndrome (Poldre). 1987;157:1267

Head circumference

Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements (Vintzileos et al). 1987;157:410-4

Heart block

Congenital heart block and cardiac anomalies in the absence of maternal connective tissue disease (Shenker et al). 1987;157:248-53

Heart disease

Fetal echocardiographic screening for congenital heart disease: the importance of the four-chamber view (Copel et al). 1987;157:648-55

Heart rate; *see* **Fetal monitoring**

Heat flux

Scalp heat flux and its relationship to scalp blood pH of the fetus (Rudelstorfer et al). 1987;157:372-7

HELLP syndrome

Haptoglobin helps diagnose the HELLP syndrome (Poldre). 1987;157:1267

HELLP syndrome (Gerace) (Letter); (Sibai) (Reply). 1987;157:1015-6

Hemarthrosis

Factor XI deficiency presenting as hemarthrosis during pregnancy (Musclow et al). 1987;157:178-9

Hemifacial microsomia

Possible vascular disruptive origin of hemifacial microsomia? (Schinzel) (Letter). 1987;157:1319

Hemodynamics

Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep (Harake et al). 1987;157:1003-8

Hemoglobin

Intrapartum use of fetal scalp hematocrit in the diagnosis of profound fetal anemia caused by fetomaternal hemorrhage (Herman et al). 1987;157:1182-3

Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short-and long-term metabolic control in gestational diabetes (Brustman et al). 1987;157:699-703

Hemolysis

The origin of increased serum iron in pregnancy-induced hypertension (Samuels et al). 1987;157:721-5

Haptoglobin helps diagnose the HELLP syndrome (Poldre). 1987;157:1267

HELLP syndrome (Gerace) (Letter); (Sibai) (Reply). 1987;157:1015-6

Hemolytic disease of newborn; *see also* **Hydrops fetalis**

Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops (Socol et al). 1987;157:1369-75

Hemorrhage; *see also* **Bleeding**

Fetal death: diagnosis and management (Pitkin). 1987;157:583-9

Fetal-induced trauma as a cause of antepartum hemorrhage (Eden). 1987;157:830-1

Intrapartum use of fetal scalp hematocrit in the diagnosis of profound fetal anemia caused by fetomaternal hemorrhage (Herman et al). 1987;157:1182-3

Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9

Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage (Cardwell). 1987;157:358-9

Hemostasis

Preeclampsia, delivery, and the hemostatic system (Saleh et al). 1987;157:331-6

Henoch-Schönlein purpura

Pregnancy in Henoch-Schönlein purpura (Joseph et al). 1987;157:911-2

Heparin

Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8

Hepatocyte

Metabolism of lipoproteins by human fetal hepatocytes (Carr). 1987;157:1338-44

Hermaphrodite

The endocrinologic evaluation of a 45,X true hermaphrodite (Rosen et al). 1987;157:1272-3

Herpes simplex

Labial agglutination secondary to herpes simplex II infection (DeMarco et al). 1987;157:296-7

High-risk pregnancy; *see also* **Fetal biophysical profile**

Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths (Manning et al). 1987;157:880-4

Hirsutism

The endocrinologic evaluation of a 45,X true hermaphrodite (Rosen et al). 1987;157:1272-3

Histology

Histologic composition of endometrial carcinomas analyzed for steroid receptor content (Soper et al). 1987;157:26-7

HLA antigen; *see* **Human leukocyte antigen**

Homozygous α-thalassemia

Theca-lutein ovarian cysts associated with homozygous α-thalassemia (Miller et al). 1987;157:912-4

Hormone replacement therapy

In vivo and in vitro modulation of growth hormone and

prolactin of a mixed somatotropic-lactotropic pituitary microadenoma (Muechler et al). 1987;157:453-5
Modulating actions of estradiol on gonadotropin-releasing hormone–stimulated prolactin secretion in postmenopausal individuals (Christiansen et al). 1987;157:320-5

Hormones
Age-related changes in the female hormonal environment during reproductive life (Musey et al). 1987;157:312-7
Circulating neurohypophyseal hormones in anencephalic infants (Oosterbaan and Swaab). 1987;157:117-9
Preliminary observations on lactoferrin secretion in human vaginal mucus: variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae* (Cohen et al). 1987;157:1122-5
Transplacental stimulation of functional and morphologic fetal rabbit lung maturation: effect of thyrotropin-releasing hormone (Devaskar et al). 1987;157:460-4

Human chorionic gonadotropin; *see* **Gonadotropins**

Human immunodeficiency virus
Isolation of acquired immunodeficiency syndrome virus from the placenta (Hill et al). 1987;157:10-1

Human leukocyte antigen
HLA antigen expression and induction by γ-interferon in cultured human trophoblasts (Feinman et al). 1987;157:1429-34
Human leukocyte antigen associated with endometrial carcinoma with a new classification of endometrial carcinoma based on its etiology (Kanuma and Igarashi). 1987;157:427-32
Recurrent spontaneous abortion and human leukocyte antigen DRwB (Takamizawa et al) (Letter). 1987;157:514-5

Human menopausal gonadotropin
Follicle-stimulating hormone is the main determinant of follicular recruitment and development in ovulation induction with human menopausal gonadotropin (Vermesh and Kletzky). 1987;157:1397-402

Hyaline membrane disease
Cholesteryl palmitate as a predictor of fetal lung maturity (Ludmir et al). 1987;157:84-8
Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984 (Ohlsson et al). 1987;157:443-5
Transplacental stimulation of functional and morphologic fetal rabbit lung maturation: effect of thyrotropin-releasing hormone (Devaskar et al). 1987;157:460-4

Hydatidiform mole
Hydatidiform mole: cytogenetically unusual cases and their implications for the present classification (Vejerslev et al). 1987;157:180-4 (Curr. dev.)
Partial hydatidiform moles: deoxyribonucleic acid content and course (David et al). 1987;157:969-73

Hydramnios
Genetic implications of idiopathic hydramnios (Landy et al). 1987;157:114-7

Hydronephrosis
The nonpredictive value of fetal urinary electrolytes: preliminary report of outcomes and correlation with pathologic diagnosis (Wilkins et al). 1987;157:694-8
Renal arterial blood flow velocity waveforms in pregnant women (Hata et al). 1987;157:1269-71

Hydrops fetalis
Insufficient transplacental digoxin transfer in severe hydrops fetalis (Younis and Granat). 1987;157:1268-9
Percutaneous ultrasound-guided fetal blood sampling in the management of nonimmune hydrops fetalis (Hsieh et al). 1987;157:44-9
Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops (Socol et al). 1987;157:1369-75
Successful fetal therapy for cystic adenomatoid malformation associated with second-trimester hydrops (Clark et al). 1987;157:294-5
Ultrasound evaluation of tachycardia-induced hydrops in the fetal lamb (Nimrod et al). 1987;157:655-9

Hydrostatic pressure
Calculated capillary hydrostatic pressure in normal pregnancy and preeclampsia (Øian and Maltau). 1987;157:102-6

Hydrothorax
Fetal pulmonary hypoplasia with hydrothorax (Murayama et al). 1987;157:119-20

Hymen
Configuration of the prepubertal hymen (Pokorny). 1987;157:950-6

Hyperimmunoglobulinemia E
Hyperimmunoglobulinemia E and pregnancy: a case report (Lindenbaum et al). 1987;157:1273-4

Hyperprolactinemia
Evidence that estrogen may be a key factor in hyperprolactinemic anovulation: a case report (Sakamoto et al). 1987;157:318-9

Hyperreactio luteinalis
Human chorionic gonadotropin, estriol, and testosterone changes in two pregnancies with hyperreactio luteinalis (Muechler et al). 1987;157:1126-8

Hypertension; *see also* **Blood pressure**
Beneficial effects of U-63,557A, a thromboxane synthetase inhibitor, in an ovine model of pregnancy-induced hypertension (Keith et al). 1987;157:199-203
A classification of hypertension in pregnancy based on Doppler velocimetry (Ducey et al). 1987;157:680-5
Hypertensive diseases of pregnancy: nulliparity, primiparity, multiparity, and gravidity (Reamy) (Letter); (Gleicher) (Reply). 1987;157:1012
Increased fetoplacental active renin production in pregnancy-induced hypertension (Brar et al). 1987;157:363-7
The origin of increased serum iron in pregnancy-induced hypertension (Samuels et al). 1987;157:721-5

Hypoglycemia
Hypoglycemia: How little is too much for the embryo? (Sadler and Hunter). 1987;157:190-3

Hypokalemia
Experiences with gossypol as a male pill (Liu et al). 1987;157:1079-81

Hypoplasia; *see* **Pulmonary hypoplasia**

Hypotension
Profound hypotension complicating magnesium therapy (Frohlich) (Letter). 1987;157:513

Hypoxemia; *see also* **Hypoxia**
The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation (Towell et al). 1987;157:1550-7

Hypoxia; *see also* **Hypoxemia**
Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons (Howard et al). 1987;157:1261-6

Stereotypic behavioral response of rat fetuses to acute hypoxia is altered by maternal alcohol consumption (Smotherman and Robinson). 1987;157:982-6

Hyskon
Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8

Hysterectomy
Single-dose prophylaxis for vaginal and abdominal hysterectomy (Hemsell et al). 1987;157:498-501

Hysterosalpingogram
Hysterosalpingographic evaluation of tubal patency after ectopic pregnancy (Mitchell et al). 1987;157:618-22

I

Ibuprofen
Cyclooxygenase inhibition reduces placental transfer: reversal by carbacyclin (Kuhn and Stuart). 1987;157:194-8

Idiopathic thrombocytopenic purpura
Validity of intrapartum diagnosis of fetal thrombocytopenia (Christiaens and Helmerhorst). 1987;157:864-5

Iliohypogastric nerve
Nerve entrapment after Pfannenstiel incision (Sippo et al). 1987;157:420-1

Imaging; *see also* **Magnetic resonance imaging; Ultrasound**
Diagnostic imaging of gynecologic tumors with the monoclonal antibody 791T/36 (Powell et al). 1987;157:28-34

Immunization
Recent developments in immunocontraception (Talwar and Gaur). 1987;157:1075-8
Varicella zoster antibody testing in the care of pregnant women exposed to varicella (McGregor et al). 1987;157:281-4

Immunoassay
Natural family planning (Brown et al). 1987;157:1082-9

Immunocontraception
Recent developments in immunocontraception (Talwar and Gaur). 1987;157:1075-8

Immunofluorescence
Detection of *Chlamydia trachomatis* cervical infection: a comparison of Papanicolaou and immunofluorescent staining with cell culture (Quinn et al). 1987;157:394-9

Immunoglobulin E
Hyperimmunoglobulinemia E and pregnancy: a case report (Lindenbaum et al). 1987;157:1273-4

Immunoglobulin M
A pregnant woman with a high level of naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii* (Konishi). 1987;157:832-3

Immunohistochemistry
Adenosquamous carcinoma of the fallopian tube: a clinicopathologic case report with verification of the diagnosis by immunohistochemical and ultrastructural studies (Moore et al). 1987;157:903-5
Endometrial adenocarcinoma estrogen receptor content: association of clinicopathologic features with immunohistochemical analysis compared with standard biochemical methods (Mutch et al). 1987;157:924-31
Ovarian prostaglandin synthase: immunohistochemical localization in the rat (Curry et al). 1987;157:537-43

Immunoreactivity
Digoxin-like immunoreactive substance in fetuses with and without cardiac pathology (Weiner et al). 1987;157:368-71
Digoxin-like immunoreactive substance in pregnancy (Gonzalez et al). 1987;157:660-4

Immunosuppression
Suppression of lymphocyte reactivity in vitro by supernatants of explants of human endometrium (Wang et al). 1987;157:956-63

Implantation
Disruption of embryonic and fetal development due to preimplantation chemical insults: a critical review (Iannaccone et al). 1987;157:476-84 (Curr. dev.)

In vitro fertilization; *see* **Fertilization**

Incompetent cervix
Decreased elastic fibers and desmosine content in incompetent cervix (Leppert et al). 1987;157:1134-9
Management of the diethylstilbestrol-exposed pregnant patient: a prospective study (Ludmir et al). 1987;157:665-9

Incontinence
Urogynecology: comments and caveats (Thiede and Saini). 1987;157:563-8

Indomethacin
Cyclooxygenase inhibition reduces placental transfer: reversal by carbacyclin (Kuhn and Stuart). 1987;157:194-8
Treatment of polyhydramnios with prostaglandin synthetase inhibitor (indomethacin) (Cabrol et al). 1987;157:422-6

Infection
Arachidonate lipoxygenase metabolites in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1454-60
Breast galactography in the treatment of infected cysts (Tihansky and DiBlasio). 1987;157:1278-80
Careful surgical technique can reduce infectious morbidity after cesarean section (Lyon and Richardson). 1987;157:557-62
Changing concepts in obstetric antibiotic prophylaxis (Galask). 1987;157:491-7
Colposcopic correlates of cervical papillomavirus infection (Follen et al). 1987;157:809-14
Correlation between chlamydia infection and clinical evaluation, vaginal wet smear, and cervical swab test in female adolescents (Thejls et al). 1987;157:974-6
Cost-effective use of antibiotic prophylaxis for cesarean section (Ford et al). 1987;157:506-10
Detection of *Chlamydia trachomatis* cervical infection: a comparison of Papanicolaou and immunofluorescent staining with cell culture (Quinn et al). 1987;157:394-9
Haemophilus influenzae infection and midtrimester abortion (Amstey and Ogden) (Letter). 1987;157:518
Infection and labor: the detection of endotoxin in amniotic fluid (Romero et al). 1987;157:815-9
Labial agglutination secondary to herpes simplex II infection (DeMarco et al). 1987;157:296-7
Necrotizing fasciitis of the vulva (Roberts). 1987;157:568-71
Preliminary observations on lactoferrin secretion in human vaginal mucus: variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae* (Cohen et al). 1987;157:1122-5
Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1461-7
Reduction of group B streptococcal maternal and neonatal infections in preterm pregnancies with premature rupture of membranes through a rapid identification test (Morales and Lim). 1987;157:13-6

Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984 (Ohlsson et al). 1987;157:443-5
A screening test (GBS-Test) for urogenital carriage of group B streptococci (Christensen and Christensen). 1987;157:341-2
Single-agent therapy for women with acute polymicrobial pelvic infections (Hemsell et al). 1987;157:488-90
Single-dose prophylaxis for vaginal and abdominal hysterectomy (Hemsell et al). 1987;157:498-501
Single-dose versus multiple-dose piiperacillin prophylaxis in primary cesarean operation (Gall and Hill). 1987;157:502-6
Symposium on infection: a complication of obstetric and gynecologic surgery. 1987;157:485-512
Symposium on infection: a complication of obstetric and gynecologic surgery: questions from the audience. 1987;157:511-2
Treatment and prevention of female pelvic infection: the quest for single-agent therapy (Cunningham). 1987;157:485-8
The use and limitations of endocervical Gram stains and mucopurulent cervicitis as predictors for *Chlamydia trachomatis* in female adolescents (Moscicki et al). 1987;157:65-71

Infertility; *see also* **Fertilization**
Contraceptive use, pelvic inflammatory disease, and fertility problems among American women: 1982 (Aral et al). 1987;157:59-64
Local peritoneal factors: their role in infertility associated with endometriosis (Awadalla et al). 1987;157:1207-14
The morphologic effect of short-term medical therapy of endometriosis (Brosens et al). 1987;157:1215-21
Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8
Primary cesarean section and subsequent fertility (LaSala and Berkeley). 1987;157:379-83
Seminal plasma proteins of fertile and infertile men analyzed by two-dimensional electrophoresis (Ayyagari et al). 1987;157:1528-33

Inositol trisphosphate
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)

Insulin; *see also* **Diabetes mellitus**
Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys (Billiar et al). 1987;157:1297-302
The effect of insulin on aromatase activity in isolated human endometrial glands and stroma (Randolph et al). 1987;157:1534-9
Gestational diabetes: insulin requirements in pregnancy (Langer et al). 1987;157:669-75
Gestational diabetes: maternal response to diet and insulin therapy as reflected by glycosylated hemoglobin concentration (Grandis et al). 1987;157:1118-21
Growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels compared in premature, small, average birth weight, and large infants (Samaan et al). 1987;157:1524-8
Influence of the menstrual cycle on glucose tolerance and insulin secretion (Bonora et al). 1987;157:140-1
Insulin resistance and abnormal ovarian responses to human chorionic gonadotropin in chronically anovulatory women (Kustin et al). 1987;157:1468-73
Postprandial walking exercise in pregnant insulin-dependent (type 1) diabetic women: reduction of plasma lipid levels but absence of a significant effect on glycemic control (Hollingsworth and Moore). 1987;157:1359-63
Restriction fragment length polymorphisms of the insulin gene hypervariable region in gestational onset diabetes mellitus (Ober et al). 1987;157:1364-8

γ-Interferon
HLA antigen expression and induction by γ-interferon in cultured human trophoblasts (Feinman et al). 1987;157:1429-34

Interleukins
Enhancement of maternal and neonatal natural killer cell activity with interleukin-2 (Baker et al). 1987;157:780-1
Local peritoneal factors: their role in infertility associated with endometriosis (Awadalla et al). 1987;157:1207-14

Intestinal obstruction
Mechanical intestinal obstruction in patients with gynecologic disease: a review of 368 patients (Krebs and Goplerud). 1987;157:577-83

Intracranial hemorrhage
Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9

Intraepithelial neoplasia; *see* **Cervical intraepithelial neoplasia**

Intrapartum fetal monitoring; *see* **Fetal monitoring**

Intrauterine device
Contraception: an overview (Hammerstein). 1987;157:1020-3

Intrauterine fetal resuscitation
A study on intrauterine fetal resuscitation with terbutaline (Patriarco et al). 1987;157:384-7

Intrauterine growth retardation; *see also* **Fetal growth**
Caffeine-cigarette interaction on fetal growth (Beaulac-Baillargeon and Desrosiers). 1987;157:1236-40
Chorionic villus sampling is associated with normal fetal growth (Williams et al). 1987;157:708-12
Cord sampling for the evaluation of oxygenation and acid-base balance in human growth-retarded fetuses (Pardi et al). 1987;157:1221-8
Fetal cerebellar growth unaffected by intrauterine growth retardation: a new parameter for prenatal diagnosis (Reece et al). 1987;157:632-8
Fetal growth retardation as a complication of pregnancy in patients with neurofibromatosis (Blickstein and Lancet). 1987;157:343
Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes (Shinozuka et al). 1987;157:1140-5
Genetic heterogeneity of prematurity and intrauterine growth retardation: clues from the Old Order Amish (Khoury and Cohen). 1987;157:400-10
Heart rate variation and movement incidence in growth-retarded fetuses: the significance of antenatal late heart rate decelerations (Bekedam et al). 1987;157:126-33
In utero ponderal index as a prognostic factor in the evaluation of intrauterine growth retardation (Yagel et al). 1987;157:415-9
Neonatal morphometrics and perinatal outcome: Who is growth retarded? (Patterson and Pouliot). 1987;157:691-3
Pregnancy among obstetricians: a comparison of births before, during, and after residency (Grunebaum et al). 1987;157:79-83

Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole (Wallenburg and Rotmans). 1987;157:1230-5
Recurrent small for gestational age birth: perinatal risks and outcomes (Wolfe et al). 1987;157:288-93
The relationship between cadmium, zinc, and birth weight in pregnant women who smoke (Kuhnert et al). 1987;157:1247-51
Sonographic amniotic fluid measurement and fetal growth retardation: a reappraisal (Patterson et al). 1987;157:1406-10

Intrauterine pressure
Simultaneous objective and subjective evaluation of meclofenamate sodium in the treatment of primary dysmenorrhea (Smith and Powell). 1987;157:611-8

Intrauterine procedures; *see also specific procedure*
The use of fetal neuromuscular blockade during intrauterine procedures (Moise et al). 1987;157:874-9
Technical aspects of intravascular intrauterine transfusions: lessons learned from thirty-three procedures (Berkowitz et al). 1987;157:4-9 (Clin. opinion)

Intravascular needle puncture
In vitro training model for diagnostic and therapeutic fetal intravascular needle puncture (Timor-Tritsch and Yeh). 1987;157:858-9

Involution
Abdominal pregnancy: magnetic resonance identification with ultrasonographic follow-up of placental involution (Spanta et al). 1987;157:887-9

Iron
The origin of increased serum iron in pregnancy-induced hypertension (Samuels et al). 1987;157:721-5
Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy (Schifman et al). 1987;157:304-7

Isoimmunization; *see also* **Hydrops fetalis**
Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops (Socol et al). 1987;157:1369-75

Items
Items. 1987;157:228, 520, 788, 1018, 1324, 1562

K

Karyotype
Fetal death: diagnosis and management (Pitkin). 1987;157:583-9
First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions (Strobino and Pantel-Silverman). 1987;157:1150-4

L

Labial agglutination
Labial agglutination secondary to herpes simplex II infection (DeMarco et al). 1987;157:296-7

Labor; *see also* **Labor, premature; Labor complications**
Estrogen, progesterone, prolactin, prostaglandin E_2, prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ gradients across the uterus in women in labor and not in labor (Davidson et al). 1987;157:54-8
Fetal heart rate response to vibratory acoustic stimulation predicts fetal pH in labor (Edersheim et al). 1987;157:1557-60
In vitro conversion of pregnenolone to progesterone in human term placenta and fetal membranes before and after onset of labor (Khan-Dawood). 1987;157:1333-7
Influence of umbilical vein administration of oxytocin on the third stage of labor: a randomized, double-blind, placebo-controlled study (Chestnut and Wilcox). 1987;157:160-2
Neonatal seizures after cesarean delivery: higher risk with labor (Spellacy et al). 1987;157:377-9
The normal parturient's admission temperature (Acker et al). 1987;157:308-11
Obstetric outcome of patients with a previous episode of spurious labor (Arulkumaran et al). 1987;157:17-20
Previous cesarean delivery and trial of labor (Fribourg) (Letter); (Finley) (Reply). 1987;157:1014-5
Uterine and umbilical artery velocimetry during normal labor (Fleischer et al). 1987;157:40-3
When does a statistical fact become an ethical imperative? (Silver and Minogue). 1987;157:229-33 (Clin. opinion)

Labor, premature
Arachidonate lipoxygenase metabolites in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1454-60
Effects of the antiprogesterone RU 486 on preterm birth in the rat (Garfield et al). 1987;157:1281-5
Increased boy-to-girl ratio in women with preterm labor beginning with contractions (Seki and Kato) (Letter). 1987;157:215-6
The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor (Besinger et al). 1987;157:753-7
A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor (Iams et al). 1987;157:638-43
A prospective randomized study of the aggressive management of early labor (Cohen et al). 1987;157:1174-7
Risk scoring for preterm labor: Where do we go from here? (Main and Gabbe). 1987;157:789-93 (Clin. opinion)

Labor complications
Early third-trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term (Bochner et al). 1987;157:703-8
Infection and labor: the detection of endotoxin in amniotic fluid (Romero et al). 1987;157:815-9
Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1461-7

Lactate
Cord sampling for the evaluation of oxygenation and acid-base balance in human growth-retarded fetuses (Pardi et al). 1987;157:1221-8
The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation (Towell et al). 1987;157:1550-7

Lactation; *see* **Breast-feeding**

Lactoferrin
Preliminary observations on lactoferrin secretion in human vaginal mucus: variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae* (Cohen et al). 1987;157:1122-5

Langerhans' cell
Langerhans' cells in the cervix (Hawthorn and MacLean) (Letter); (McArdle) (Reply). 1987;157:213-5

Laparoscopy
Carbon dioxide laser laparoscopy performed with a flexible fiber in humans (Baggish et al). 1987;157:1129-33

Unintended laparotomy associated with laparoscopic tubal sterilization (Franks et al). 1987;157:1102-5

Laparotomy
Unintended laparotomy associated with laparoscopic tubal sterilization (Franks et al). 1987;157:1102-5

Laser
Carbon dioxide laser laparoscopy performed with a flexible fiber in humans (Baggish et al). 1987;157:1129-33
Labial agglutination secondary to herpes simplex II infection (DeMarco et al). 1987;157:296-7
Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia (Anderson) (Letter); (Baggish) (Reply). 1987;157:1322-3

Leukocyte
HLA antigen expression and induction by γ-interferon in cultured human trophoblasts (Feinman et al). 1987;157;1429-34
Human leukocyte antigen associated with endometrial carcinoma with a new classification of endometrial carcinoma based on its etiology (Kanuma and Igarashi). 1987;157:427-32
Leukocyte esterase activity in human amniotic fluid for the rapid detection of chorioamnionitis (Hoskins et al). 1987;157:730-2
Recurrent spontaneous abortion and human leukocyte antigen DRw8 (Takamizawa et al) (Letter). 1987;157: 514-5

Levonorgestrel
A new delivery system for contraceptive steroids (Segal). 1987;157:1090-2

Liley chart
Have Liley charts outlived their usefulness? (Duhring) (Letter); (Nicolaides) (Reply). 1987;157:1011-2

***Limulus* amebocyte**
Infection and labor: the detection of endotoxin in amniotic fluid (Romero et al). 1987;157:815-9

Lipids
Postprandial walking exercise in pregnant insulin-dependent (type 1) diabetic women: reduction of plasma lipid levels but absence of a significant effect on glycemic control (Hollingsworth and Moore). 1987;157: 1359-63

Lipoprotein
The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly (Carr and Parker). 1987;157:1351-5
Metabolism of lipoproteins by human fetal hepatocytes (Carr). 1987;157:1338-44

β-Lipotropin
Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: changes during the menstrual cycle (Petraglia et al). 1987;157:142-6

Listeria
Fetal death: diagnosis and management (Pitkin). 1987;157:583-9

Low birth weight
Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes (Shinozuka et al). 1987;157:1140-5
Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9

Low-density lipoprotein
The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly (Carr and Parker). 1987;157:1351-5

Lung
Cholesteryl palmitate as a predictor of fetal lung maturity (Ludmir et al). 1987;157:84-8
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91
Effects of norepinephrine on lung fluid flow rate in the chronically catheterized fetal lamb (Higuchi et al). 1987;157:986-90
Transplacental stimulation of functional and morphologic fetal rabbit lung maturation: effect of thyrotropin-releasing hormone (Devaskar et al). 1987;157:460-4

Lupus erythematosus
Fetal death: diagnosis and management (Pitkin). 1987;157:583-9
Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant (Druzin et al). 1987;157:1503-10

Luteinizing hormone
Acute effects of exercise on plasma catecholamines in sedentary and athletic women with normal and abnormal menses (Chin et al). 1987;157:938-44

Lymphocyte
Lymphocyte-reactive antibodies and recurrent early pregnancy failure (Biddle et al) (Letter). 1987;157:785-6
Suppression of lymphocyte reactivity in vitro by supernatants of explants of human endometrium (Wang et al). 1987;157:956-63

M

Macrosomia
Early third-trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term (Bochner et al). 1987;157:703-8

Magnesium sulfate
Cerebrospinal fluid levels of magnesium in patients with preeclampsia after treatment with intravenous magnesium sulfate: a preliminary report (Thurnau et al). 1987;157:1435-8
The effect of oxytocin infusion on the pharmacokinetics of intramuscular magnesium sulfate therapy (Bloss et al). 1987;157:156-60
Magnesium and zinc status during the menstrual cycle (Deuster et al). 1987;157:964-8
Profound hypotension complicating magnesium therapy (Frohlich) (Letter). 1987;157:513

Magnetic resonance imaging
Abdominal pregnancy: magnetic resonance identification with ultrasonographic follow-up of placental involution (Spanta et al). 1987;157:887-9
Magnetic resonance imaging of monkey placenta with manganese enhancement (Kay et al). 1987;157:185-9

Male contraception
Experiences with gossypol as a male pill (Liu et al). 1987;157:1079-81

Malignancy; *see also* **Carcinoma; Neoplasia; Tumor**
Serum copper level in gynecologic malignancies (Margalioth et al). 1987;157:93-6

Mammography
Breast galactography in the treatment of infected cysts (Tihansky and DiBlasio). 1987;157:1278-80

Manganese
Magnetic resonance imaging of monkey placenta with manganese enhancement (Kay et al). 1987;157: 185-9

Maternal age
Pregnancy outcome in medically complicated and uncomplicated patients aged 40 years or older (Lehmann and Chism). 1987;157:738-42
Maternal ambulation
The effects of maternal ambulation on the nonstress test (Devoe et al). 1987;157:240-4
Maternal mortality
A thirty-year review of maternal mortality in Oklahoma, 1950 through 1979 (Moses et al). 1987;157:1189-94
Maternotrophoblastic border
Echocardiographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy (Hustin and Schaaps). 1987;157:162-8
Mean arterial pressure
Blood pressure in the midtrimester and future eclampsia (Chesley and Sibai). 1987;157:1258-61
Meclofenamate
Simultaneous objective and subjective evaluation of meclofenamate sodium in the treatment of primary dysmenorrhea (Smith and Powell). 1987;157:611-8
Treatment of menorrhagia with meclofenamate sodium (Vargyas et al). 1987;157:944-50
Meconium
Effects of alterations of zinc-to-phosphorus ratios and meconium content on Group B *Streptococcus* growth in human amniotic fluid in vitro (Hoskins et al). 1987;157:770-3
Membranes; *see* **Fetal membranes**
Menopause
Continuous estrogen/progestin therapy in menopause (Prough et al). 1987;157:1449-53
Follicle-stimulating hormone is the main determinant of follicular recruitment and development in ovulation induction with human menopausal gonadotropin (Vermesh and Kletzky). 1987;157:1397-402
Menstrual and reproductive factors and the risk of myocardial infarction in women under 55 years of age (La Vecchia et al). 1987;157:1108-12
Modulating actions of estradiol on gonadotropin-releasing hormone–stimulated prolactin secretion in postmenopausal individuals (Christiansen et al). 1987;157:320-5
Menorrhagia
Treatment of menorrhagia with meclofenamate sodium (Vargyas et al). 1987;157:944-50
Menstruation
Acute effects of exercise on plasma catecholamines in sedentary and athletic women with normal and abnormal menses (Chin et al). 1987;157:938-44
Influence of the menstrual cycle on glucose tolerance and insulin secretion (Bonora et al). 1987;157:140-1
Magnesium and zinc status during the menstrual cycle (Deuster et al). 1987;157:964-8
Menstrual and reproductive factors and the risk of myocardial infarction in women under fifty-five years of age (La Vecchia et al). 1987;157:1108-12
Preliminary observations on lactoferrin secretion in human vaginal mucus: variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae* (Cohen et al). 1987;157:1122-5
Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: changes during the menstrual cycle (Petraglia et al). 1987;157:142-6
Mesometrium
Characteristics of the electromyogram recorded from the mesometrium of the pregnant ewe from 106 days' gestation to delivery: similarities with and differences from the electromyogram obtained from the myometrium (Figueroa et al). 1987;157:991-8
Metabolism
The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly (Carr and Parker). 1987;157:1351-5
Cerebral oxidative metabolism in fetal sheep with multiple-dose ethanol infusion (Richardson et al). 1987;157:1496-502
A hypothesis concerning the general basis of organogenetic congenital anomalies (Naftolin et al). 1987;157:1-4 (Clin. opinion)
Metabolism of lipoproteins by human fetal hepatocytes (Carr). 1987;157:1338-44
The significance of one abnormal glucose tolerance test value on adverse outcome in pregnancy (Langer et al). 1987;157:758-63
Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua (Challis and Vaughan). 1987;157:1474-81
Metaplasia
Endometrial metaplasia associated with endometrial adenocarcinoma (Andersen et al). 1987;157:597-604
Methimazole
Antithyroid drugs: to breast-feed or not to breast-feed (Cooper). 1987;157:234-5 (Clin. opinion)
Methionine enkephalin
Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: changes during the menstrual cycle (Petraglia et al). 1987;157:142-6
Methotrexate
Treatment of nonmetastatic gestational trophoblastic disease with oral methotrexate (Barter et al). 1987;157:1166-8
Metoclopramide
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91
Mexiletine
Mexiletine use in pregnancy and lactation (Lownes and Ives). 1987;157:446-7
Microadenoma
In vivo and in vitro modulation of growth hormone and prolactin of a mixed somatotropic-lactotropic pituitary microadenoma (Muechler et al). 1987;157:453-5
Microsomia
Possible vascular disruptive origin of hemifacial microsomia? (Schinzel) (Letter). 1987;157:1319
Microsurgery
Conservative surgical management of isthmic ectopic pregnancies (Smith et al). 1987;157:604-10
Monitoring; *see* **Fetal monitoring**
Monoamniotic twins
Antenatal diagnosis and management of monoamniotic twins (Rodis et al). 1987;157:1255-7
Monoclonal antibody
Diagnostic imaging of gynecologic tumors with the monoclonal antibody 791T/36 (Powell et al). 1987;157:28-34
Morality
Morality of induced abortion and freedom of choice (Fribourg) (Letter); (Hill) (Reply). 1987;157:215

Morbidity
Careful surgical technique can reduce infectious morbidity after cesarean section (Lyon and Richardson). 1987;157:557-62
Cocaine use during pregnancy: adverse perinatal outcome (MacGregor et al). 1987;157:686-90
Leukocyte esterase activity in human amniotic fluid for the rapid detection of chorioamnionitis (Hoskins et al). 1987;157:730-2
Prophylactic antibiotics for cesarean delivery: a simple cost-effective strategy for prevention of postoperative morbidity (Duff). 1987;157:794-8 (Clin. opinion)
Regional program for prevention of premature birth in northwestern North Carolina (Meis et al). 1987;157:550-6

Morphometrics
Neonatal morphometrics and perinatal outcome: Who is growth retarded? (Patterson and Pouliot). 1987;157:691-3

Mortality; *see also* **Death**
Evaluation of a perinatal grief support team (Lake et al). 1987;157:1203-6
Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984 (Ohlsson et al). 1987;157:443-5
A thirty-year review of maternal mortality in Oklahoma, 1950 through 1979 (Moses et al). 1987;157:1189-94

Mucus
Preliminary observations on lactoferrin secretion in human vaginal mucus: variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae* (Cohen et al). 1987;157:1122-5

Mycotic aneurysm
Mycotic aneurysms in pregnancy (Boike et al). 1987;157:340-1

Myocardial infarction
Coagulation effects of oral contraception (Bonnar). 1987;157:1042-8
Menstrual and reproductive factors and the risk of myocardial infarction in women under fifty-five years of age (La Vecchia et al). 1987;157:1108-12
Myocardial infarction in the puerperium (Feldman) (Letter); (Iffy et al) (Reply). 1987;157:515-6

Myometrium
Changes in cyclic adenosine monophosphate–phosphodiesterase activity in nonpregnant and pregnant human myometrium (Kofinas et al). 1987;157:733-8
Characteristics of the electromyogram recorded from the mesometrium of the pregnant ewe from 106 days' gestation to delivery: similarities with and differences from the electromyogram obtained from the myometrium (Figueroa et al). 1987;157:991-8
The effects of aminophylline and nifedipine on contractility of isolated pregnant human myometrium (Bird et al). 1987;157:171-7
Effects of the antiprogesterone RU 486 on preterm birth in the rat (Garfield et al). 1987;157:1281-5
Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs (Elger et al). 1987;157:1065-74
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)

Myosin
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)

N

Natriuretic peptide
Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus (Ross et al). 1987;157:1292-7

Natural family planning
Natural family planning (Brown et al). 1987;157:1082-9

Natural killer cells
Enhancement of maternal and neonatal natural killer cell activity with interleukin-2 (Baker et al). 1987;157:780-1

Necrotizing fasciitis
Necrotizing fasciitis of the vulva (Roberts). 1987;157:568-71

Needle puncture
Cervical endometriosis: facilitated diagnosis by fine needle aspiration cytologic testing (Veiga-Ferreira et al). 1987;157:849-56
In vitro training model for diagnostic and therapeutic fetal intravascular needle puncture (Timor-Tritsch and Yeh). 1987;157:858-9

Negroid race
Maternal serum α-fetoprotein screening. III. Pitfalls in evaluating black gravid women (Macri et al). 1987;157:820-2

Neisseria gonorrhoeae
Preliminary observations on lactoferrin secretion in human vaginal mucus: variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae* (Cohen et al). 1987;157:1122-5

Neonate
Abnormalities in platelet antiglobulin tests in preeclamptic mothers and their neonates (Samuels et al). 1987;157:109-13
The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly (Carr and Parker) 1987;157:1351-5
Circulating neurohypophyseal hormones in anencephalic infants (Oosterbaan and Swaab). 1987;157:117-9
Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes (Shinozuka et al). 1987;157:1140-5
Growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels compared in premature, small, average birth weight, and large infants (Samaan et al). 1987;157:1524-8
Neonatal auditory acuity following in utero vibratory acoustic stimulation (Ohel et al). 1987;157:440-1
Neonatal morphometrics and perinatal outcome: Who is growth retarded? (Patterson and Pouliot). 1987;157:691-3
Neonatal seizures after cesarean delivery: higher risk with labor (Spellacy et al). 1987;157:377-9
Reduction of group B streptococcal maternal and neonatal infections in preterm pregnancies with premature rupture of membranes through a rapid identification test (Morales and Lim). 1987;157:13-6
Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984 (Ohlsson et al). 1987;157:443-5

Neoplasia; *see also* **Carcinoma; Malignancy; Tumor**
Colposcopic correlates of cervical papillomavirus infection (Follen et al). 1987;157:809-14

Evaluation of the atypical Pap smear (Jones et al). 1987;157:544-9
Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia (Anderson) (Letter); (Baggish) (Reply). 1987;157:1322-3
Multiple primary gynecologic neoplasms (Rose et al). 1987;157:261-7
The results of cryosurgical treatment of cervical intraepithelial neoplasia at one, five, and ten years (Benedet et al). 1987;157:268-73

Nerve entrapment
Nerve entrapment after Pfannenstiel incision (Sippo et al). 1987;157:420-1

Neurofibromatosis
Fetal growth retardation as a complication of pregnancy in patients with neurofibromatosis (Blickstein and Lancet). 1987;157:343

Neurohypophyseal hormones
Circulating neurohypophyseal hormones in anencephalic infants (Oosterbaan and Swaab). 1987;157:117-9

Neuromuscular blockade
The use of fetal neuromuscular blockade during intrauterine procedures (Moise et al). 1987;157:874-9

Neutrophil
Host defense during pregnancy: neutrophil chemotaxis and adherence (Krause et al). 1987;157:274-80

Newborn; *see* **Neonate**

Nicardipine
Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes (Ducsay et al). 1987;157:1482-6

Nifedipine
The effects of aminophylline and nifedipine on contractility of isolated pregnant human myometrium (Bird et al). 1987;157:171-7
Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep (Harake et al). 1987;157:1003-8

Nipple stimulation
Breast stimulation test and oxytocin challenge test in fetal surveillance: a prospective randomized study (Lipitz et al). 1987;157:1178-81
A prospective comparative study of the extended nonstress test and the nipple stimulation contraction stress test (Devoe et al). 1987;157:531-7
A prospective evaluation of nipple stimulation techniques for contraction stress testing (Keegan et al). 1987;157:121-5

Noise
Local cerebral glucose utilization in fetal sheep exposed to noise (Abrams et al). 1987;157:456-60

Nonstress test
Antepartum predictors of fetal distress in postterm pregnancy (Bochner et al). 1987;157:353-8
Criteria for interpretation of a reactive nonstress test (Willis) (Letter); (Erskine et al) (Reply). 1987;157:213
The effects of maternal ambulation on the nonstress test (Devoe et al). 1987;157:240-4
Patterns of human fetal heart rate accelerations from 26 weeks to term (Gagnon et al). 1987;157:743-8
A prospective comparative study of the extended nonstress test and the nipple stimulation contraction stress test (Devoe et al). 1987;157:531-7

Norepinephrine
Effects of norepinephrine on lung fluid flow rate in the chronically catheterized fetal lamb (Higuchi et al). 1987;157:986-90

Norgestimate
New progestogens in oral contraceptives (Runnebaum and Rabe). 1987;157:1059-63

Nuchal fold
Soft tissue nuchal fold in the second-trimester fetus: standards for normal measurements compared with those in Down syndrome (Benacerraf and Frigoletto). 1987;157:1146-9

O

Obstetric residents
Pregnancy among obstetricians: a comparison of births before, during, and after residency (Grunebaum et al). 1987;157:79-83

Obstruction
Mechanical intestinal obstruction in patients with gynecologic disease: a review of 368 patients (Krebs and Goplerud). 1987;157:577-83

Oligohydramnios
Acute oligohydramnios in postdate pregnancy (Clement et al). 1987;157:884-6
Elevated maternal serum α-fetoprotein levels and oligohydramnios: poor prognosis for pregnancy outcome (Dyer et al). 1987;157:336-9
Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia (Johnson et al). 1987;157:764-9

Oocyte
Transurethral ultrasound-guided oocyte retrieval for in vitro fertilization (Padilla et al). 1987;157:622-6

Oral contraception
Coagulation effects of oral contraception (Bonnar). 1987;157:1042-8
Contraception: an overview (Hammerstein). 1987;157:1020-3
Hormonal contraception: benefits versus risks (Goldzieher). 1987;157:1023-8
International symposium on contraception. Part 1: oral contraception. 1987;157:1019-63
International symposium on contraception. Part 1: oral contraception: introduction (Speroff). 1987;157:1019
Menstrual and reproductive factors and the risk of myocardial infarction in women under 55 years of age (La Vecchia et al). 1987;157:1108-12
Metabolic effects of oral contraceptives (Gaspard). 1987;157:1029-41
New progestogens in oral contraceptives (Runnebaum and Rabe). 1987;157:1059-63
Phasic approach to oral contraceptives (Hale). 1987;157:1052-8
Progestogen potency in oral contraceptive pills (Gillmer). 1987;157:1048-52
Venous thrombosis in patients with cardiac pacemakers and oral contraceptive use (Rao) (Letter). 1987;157:516-7

Organogenesis
Absence of clomiphene and/or active metabolites in blood of clomiphene citrate–treated patients during organogenesis: determination by radioreceptor assay (Geier et al) (Letter). 1987;157:1009
A hypothesis concerning the general basis of organogenetic congenital anomalies (Naftolin et al). 1987;157:1-4 (Clin. opinion)

Osmotic pressure
Mechanisms for reduced colloid osmotic pressure in preeclampsia (Bhatia et al). 1987;157:106-8

Outcome of pregnancy
Cocaine use during pregnancy: adverse perinatal outcome (MacGregor et al). 1987;157:686-90
Fetal death: diagnosis and management (Pitkin). 1987;157:583-9
Hysterosalpingographic evaluation of tubal patency after ectopic pregnancy (Mitchell et al). 1987;157:618-22
Neonatal morphometrics and perinatal outcome: who is growth retarded? (Patterson and Pouliot). 1987;157:691-3
Pregnancy outcome in medically complicated and uncomplicated patients aged 40 years or older (Lehmann and Chism). 1987;157:738-42
The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor (Besinger et al). 1987;157:753-7
Recurrent small for gestational age birth: perinatal risks and outcome (Wolfe et al). 1987;157:288-93
Responsibility, obligation, and duty (Dunn). 1987;157:521-30
The significance of one abnormal glucose tolerance test value on adverse outcome in pregnancy (Langer et al). 1987;157:758-63

Ovarian carcinoma
Another look at the second-assessment procedure for ovarian epithelial carcinoma (Gallup et al). 1987;157:590-6
Cigarette smoking and the risk of female reproductive cancer (Stockwell and Lyman). 1987;157:35-40
Evaluation of serum CA 125 levels in the monitoring of ovarian cancer (Vergote et al). 1987;157:88-92

Ovarian cyst
Theca-lutein ovarian cysts associated with homozygous α-thalassemia (Miller et al). 1987;157:912-4

Ovary
Human ovarian oxytocin and steroid hormones (Auletta and Flint) (Letter); (Khan-Dawood and Dawood) (Reply). 1987;157:1010-1
Insulin resistance and abnormal ovarian responses to human chorionic gonadotropin in chronically anovulatory women (Kustin et al). 1987;157:1468-73
Ovarian prostaglandin synthase: immunohistochemical localization in the rat (Curry et al). 1987;157:537-43

Ovulation
The effects of proteolytic enzymes on in vitro ovulation in the rabbit (Yoshimura et al). 1987;157:468-75
Evidence that estrogen may be a key factor in hyperprolactinemic anovulation: a case report (Sakamoto et al). 1987;157:318-9
Follicle-stimulating hormone is the main determinant of follicular recruitment and development in ovulation induction with human menopausal gonadotropin (Vermesh and Kletzky). 1987;157:1397-402
Insulin resistance and abnormal ovarian responses to human chorionic gonadotropin in chronically anovulatory women (Kustin et al). 1987;157:1468-73
Ovarian prostaglandin synthase: immunohistochemical localization in the rat (Curry et al). 1987;157:537-43
Sonographic evaluation of the cervix during ovulation induction (Hill et al). 1987;157:1170-4

Oxygenation
Cord sampling for the evaluation of oxygenation and acid-base balance in human growth-retarded fetuses (Pardi et al). 1987;157:1221-8

Oxytocin
Breast stimulation test and oxytocin challenge test in fetal surveillance: a prospective randomized study (Lipitz et al). 1987;157:1178-81
Circulating neurohypophyseal hormones in anencephalic infants (Oosterbaan and Swaab). 1987;157:117-9
The effect of oxytocin infusion on the pharmacokinetics of intramuscular magnesium sulfate therapy (Bloss et al). 1987;157:156-60
Evaluation of 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: a new tocolytic agent (Hahn et al). 1987;157:977-82
Human ovarian oxytocin and steroid hormones (Auletta and Flint) (Letter); (Khan-Dawood and Dawood) (Reply). 1987;157:1010-1
Influence of umbilical vein administration of oxytocin on the third stage of labor: a randomized, double-blind, placebo-controlled study (Chestnut and Wilcox). 1987;157:160-2
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)

P

Pacemaker
Ultrasound evaluation of tachycardia-induced hydrops in the fetal lamb (Nimrod et al). 1987;157:655-9
Venous thrombosis in patients with cardiac pacemakers and oral contraceptive use (Rao) (Letter). 1987;157:516-7

Palsy
Erb's palsy and fetal bruising after external cephalic version: case report (Petrikovsky et al). 1987;157:258

Pancuronium
The use of fetal neuromuscular blockade during intrauterine procedures (Moise et al). 1987;157:874-9

Papanicolaou smear
Colposcopic evaluation of abnormal Papanicolaou smears in pregnancy (Benedet et al). 1987;157:932-7
Detection of *Chlamydia trachomatis* cervical infection: a comparison of Papanicolaou and immunofluorescent staining with cell culture (Quinn et al). 1987;157:394-9
Evaluation of the atypical Pap smear (Jones et al). 1987;157:544-9
A reusable plastic training model for teaching Papanicolaou smear technique (Beckmann et al). 1987;157:259-60

Papers
Papers of the Society for Gynecologic Investigation. 1987;157:1333-560

Papillomavirus
Colposcopic correlates of cervical papillomavirus infection (Follen et al). 1987;157:809-14

Paralysis
Erb's palsy and fetal bruising after external cephalic version: case report (Petrikovsky et al). 1987;157:258
The use of fetal neuromuscular blockade during intrauterine procedures (Moise et al). 1987;157:874-9

Parturition; *see also* **Delivery; Labor**
Decreased elastic fibers and desmosine content in incompetent cervix (Leppert et al). 1987;157:1134-9
The normal parturient's admission temperature (Acker et al). 1987;157:308-11

Pelvic adhesion
Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8

Pelvic infection; *see also* **Infection**
Contraceptive use, pelvic inflammatory disease, and fertility

problems among American women: 1982 (Aral et al). 1987;157:59-64
Single-agent therapy for women with acute polymicrobial pelvic infections (Hemsell et al). 1987;157:488-90
Treatment and prevention of female pelvic infection: the quest for single-agent therapy (Cunningham). 1987;157:485-8

Pemphigus
Pemphigus in pregnancy: a reevaluation of fetal risk (Metzker and Merlob) (Letter). 1987;157:1012-3

Peptides
Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: changes during the menstrual cycle (Petraglia et al). 1987;157:142-6

Periareolar sinus
Breast galactography in the treatment of infected cysts (Tihansky and DiBlasio). 1987;157:1278-80

Perinatal asphyxia
Indicators of perinatal asphyxia (Hollander et al). 1987;157:839-42
Review of causes of perinatal mortality in a regional perinatal center, 1980 to 1984 (Ohlsson et al). 1987;157:443-5

Perinatal morbidity; *see* **Morbidity**

Perinatal mortality; *see* **Mortality**

Perinatal outcome; *see* **Outcome of pregnancy**

Perinatal risk; *see* **Outcome of pregnancy**

Perineal infiltration
2-Chloroprocaine for local perineal infiltration (Philipson et al). 1987;157:1275-8

Peritoneal abnormalities
Local peritoneal factors: their role in infertility associated with endometriosis (Awadalla et al). 1987;157:1207-14

Pfannenstiel incision
Nerve entrapment after Pfannenstiel incision (Sippo et al). 1987;157:420-1

pH
Fetal heart rate response to vibratory acoustic stimulation predicts fetal pH in labor (Edersheim et al). 1987;157:1557-60
The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores (Vintzileos et al). 1987;157:627-31
Scalp heat flux and its relationship to scalp blood pH of the fetus (Rudelstorfer et al). 1987;157:372-7
Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9
Umbilical cord blood pH and Apgar score as an index of neonatal health (Josten et al). 1987;157:843-8

Pharmacokinetics
The effect of oxytocin infusion on the pharmacokinetics of intramuscular magnesium sulfate therapy (Bloss et al). 1987;157:156-60

Phosphatidylglycerol
The reliability and clinical use of a rapid phosphatidylglycerol assay in normal and diabetic pregnancies (Saad et al). 1987;157:1516-20

Phosphodiesterase
Changes in cyclic adenosine monophosphate–phosphodiesterase activity in nonpregnant and pregnant human myometrium (Kofinas et al). 1987;157:733-8

Phosphorus
Effects of alterations of zinc-to-phosphorus ratios and meconium content on group B *Streptococcus* growth in human amniotic fluid in vitro (Hoskins et al). 1987;157:770-3
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)

Piperacillin
Single-dose versus multiple-dose piperacillin prophylaxis in primary cesarean operation (Gall and Hill). 1987;157:502-6

Pituitary gland
In vivo and in vitro modulation of growth hormone and prolactin of a mixed somatotropic-lactotropic pituitary microadenoma (Muechler et al). 1987;157:453-5
Pituitary responses to synthetic corticotropin-releasing hormone: absence of modulatory effects by estrogen and progestin (Liu et al). 1987;157:1387-91

Placenta
Abdominal pregnancy: magnetic resonance identification with ultrasonographic follow-up of placental involution (Spanta et al). 1987;157:887-9
2-Chloroprocaine for local perineal infiltration (Philipson et al). 1987;157:1275-8
Cyclooxygenase inhibition reduces placental transfer: reversal by carbacyclin (Kuhn and Stuart). 1987;157:194-8
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91
The effect of smoking on placental and fetal zinc status (Kuhnert et al). 1987;157:1241-6
Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons (Howard et al). 1987;157:1261-6
In vitro conversion of pregnenolone to progesterone in human term placenta and fetal membranes before and after onset of labor (Khan-Dawood). 1987;157:1333-7
Increased fetoplacental active renin production in pregnancy-induced hypertension (Brar et al). 1987;157:363-7
Influence of umbilical vein administration of oxytocin on the third stage of labor: a randomized, double-blind, placebo-controlled study (Chestnut and Wilcox). 1987;157:160-2
Insufficient transplacental digoxin transfer in severe hydrops fetalis (Younis and Granat). 1987;157:1268-9
Isolation of acquired immunodeficiency syndrome virus from the placenta (Hill et al). 1987;157:10-1
Magnetic resonance imaging of monkey placenta with manganese enhancement (Kay et al). 1987;157:185-9
Placental bed biopsy technique and vascular lesions (Labarrere) (Letter); (Robertson) (Reply). 1987;157:1320-2
Placental glycolysis and energy metabolism in preeclampsia (Bloxam et al). 1987;157:97-101
Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole (Wallenburg and Rotmans). 1987;157:1230-5
Transplacental stimulation of functional and morphologic fetal rabbit lung maturation: effect of thyrotropin-releasing hormone (Devaskar et al). 1987;157:460-4
Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation (Trudinger et al). 1987;157:1443-8
Umbilical artery flow velocity waveforms and the placental vascular bed (McCowan et al). 1987;157:900-2
The umbilical cord twist: origin, direction, and relevance (Lacro et al). 1987;157:833-8

Placenta percreta
Acute abdomen caused by placenta percreta in the second trimester (Archer and Furlong). 1987;157:146-7

Plasma
Acute effects of exercise on plasma catecholamines in sedentary and athletic women with normal and abnormal menses (Chin et al). 1987;157:938-44
β-Adrenergic receptor agonist infusion increases plasma prostaglandin F levels in pregnant sheep (Casper and Lye). 1987;157:998-1003
Inhibitory effect of quinidine on plasma pseudocholinesterase activity in pregnant women (Kambam et al). 1987;157:897-9
Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes (Ducsay et al). 1987;157:1482-6
Mechanisms for reduced colloid osmotic pressure in preeclampsia (Bhatia et al). 1987;157:106-8
Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus (Ross et al). 1987;157:1292-7
Postprandial walking exercise in pregnant insulin-dependent (type 1) diabetic women: reduction of plasma lipid levels but absence of a significant effect on glycemic control (Hollingsworth and Moore). 1987;157:1359-63
Seminal plasma proteins of fertile and infertile men analyzed by two-dimensional electrophoresis (Ayyagari et al). 1987;157:1528-33

Platelets
Abnormalities in platelet antiglobulin tests in preeclamptic mothers and their neonates (Samuels et al). 1987;157:109-13
Increased thromboxane biosynthesis in normal pregnancy is mainly derived from platelets (Fitzgerald et al). 1987;157:325-30
Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole (Wallenburg and Rotmans). 1987;157:1230-5
Validity of intrapartum diagnosis of fetal thrombocytopenia (Christiaens and Helmerhorst). 1987;157:864-5

Pleural effusion
Pleural effusions and pulmonary hypoplasia (Castillo et al). 1987;157:1252-5

Polycystic ovary syndrome
Human chorionic gonadotropin, estriol, and testosterone changes in two pregnancies with hyperreactio luteinalis (Muechler et al). 1987;157:1126-8

Polyhydramnios
Treatment of polyhydramnios with prostaglandin synthetase inhibitor (indomethacin) (Cabrol et al). 1987;157:422-6

Polyps
Sonographic diagnosis of a bladder polyp (Jensen and Lynne). 1987;157:1168-9

Ponderal index
In utero ponderal index as a prognostic factor in the evaluation of intrauterine growth retardation (Yagel et al). 1987;157:415-9

Postdate pregnancy
Acute oligohydramnios in postdate pregnancy (Clement et al). 1987;157:884-6
Antepartum predictors of fetal distress in postterm pregnancy (Bochner et al). 1987;157:353-8
Fetal umbilical artery flow velocimetry in postdate pregnancies (Guidetti et al). 1987;157:1521-3
Umbilical cord size and amniotic fluid volume in prolonged pregnancy (Silver et al). 1987;157:716-20

Postoperative morbidity
Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8
Prophylactic antibiotics for cesarean delivery: a simple cost-effective strategy for prevention of postoperative morbidity (Duff). 1987;157:794-8 (Clin. opinion)

Preeclampsia
Abnormalities in platelet antiglobulin tests in preeclamptic mothers and their neonates (Samuels et al). 1987;157:109-13
Acute atherosis in preeclampsia: maternal determinants and fetal outcome in the presence of the lesion (Khong et al). 1987;157:360-3
Calculated capillary hydrostatic pressure in normal pregnancy and preeclampsia (Øian and Maltau). 1987;157:102-6
Cerebrospinal fluid levels of magnesium in patients with preeclampsia after treatment with intravenous magnesium sulfate: a preliminary report (Thurnau et al). 1987;157:1435-8
Digoxin-like immunoreactive substance in pregnancy (Gonzalez et al). 1987;157:660-4
The effect of oxytocin infusion on the pharmacokinetics of intramuscular magnesium sulfate therapy (Bloss et al). 1987;157:156-60
Hereditary protein C deficiency during pregnancy (Brenner et al). 1987;157:1160-1
Increased tubular enzyme excretion in preeclampsia (Goren et al). 1987;157:906-8
Mechanisms for reduced colloid osmotic pressure in preeclampsia (Bhatia et al). 1987;157:106-8
Placental glycolysis and energy metabolism in preeclampsia (Bloxam et al). 1987;157:97-101
Preeclampsia, delivery, and the hemostatic system (Saleh et al). 1987;157:331-6

Pregnancy; *see also* **Pregnancy complications**
β-Adrenergic receptor agonist infusion increases plasma prostaglandin F levels in pregnant sheep (Casper and Lye). 1987;157:998-1003
Amniotic fluid α-fetoprotein levels and pregnancy outcome (Brumfield et al). 1987;157:822-5
Anorexia nervosa, bulimia, and pregnancy (Stewart et al). 1987;157:1194-8
The "breakfast tolerance test": screening for gestational diabetes with a standardized mixed nutrient meal (Coustan et al). 1987;157:1113-7
Caffeine-cigarette interaction on fetal growth (Beaulac-Baillargeon and Desrosiers). 1987;157:1236-40
Calculated capillary hydrostatic pressure in normal pregnancy and preeclampsia (Øian and Maltau). 1987;157:102-6
The cause of uterine softening in pregnancy (Jakobovits et al) (Letter). 1987;157:1013
Changes in cyclic adenosine monophosphate–phosphodiesterase activity in nonpregnant and pregnant human myometrium (Kofinas et al). 1987;157:733-8
Characteristics of the electromyogram recorded from the mesometrium of the pregnant ewe from 106 days' gestation to delivery: similarities with and differences from the electromyogram obtained from the myometrium (Figueroa et al). 1987;157:991-8
Cord serum bromide concentration: variation and lack of association with pregnancy outcome (Miller et al). 1987;157:826-30
Digoxin-like immunoreactive substance in pregnancy (Gonzalez et al). 1987;157:660-4

Disposition of acute, multiple-dose ethanol in the near-term pregnant ewe (Brien et al). 1987;157:204-11
Echocardiographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy (Hustin and Schaaps). 1987;157:162-8
The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation (Towell et al). 1987;157:1550-7
Effect of pregnancy on surgically induced endometriosis in cynomolgus monkeys (Schenken et al). 1987;157:1392-6
Effects of aerobic and strength conditioning on pregnancy outcomes (Hall and Kaufmann). 1987;157:1199-203
The effects of aminophylline and nifedipine on contractility of isolated pregnant human myometrium (Bird et al). 1987;157:171-7
Effects of the antiprogesterone RU 486 on preterm birth in the rat (Garfield et al). 1987;157:1281-5
Effects of maternal glucose infusion on fetal acid-base status in human pregnancy (Philipson et al). 1987;157:866-73
Emotional responses of pregnant women to chorionic villi sampling or amniocentesis (Spencer and Cox). 1987;157:1155-60
Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs (Elger et al). 1987;157:1065-74
Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths (Manning et al). 1987;157:880-4
Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes (Shinozuka et al). 1987;157:1140-5
Host defense during pregnancy: neutrophil chemotaxis and adherence (Krause et al). 1987;157:274-80
Increased thromboxane biosynthesis in normal pregnancy is mainly derived from platelets (Fitzgerald et al). 1987;157:325-30
Inhibitory effect of quinidine on plasma pseudocholinesterase activity in pregnant women (Kambam et al). 1987;157:897-9
Management of the diethylstilbestrol-exposed pregnant patient: a prospective study (Ludmir et al). 1987;157:665-9
Mexiletine use in pregnancy and lactation (Lownes and Ives). 1987;157:446-7
Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep (Harake et al). 1987;157:1003-8
Observations on the log human chorionic gonadotropin–time relationship in early pregnancy and its practical implications (Kadar and Romero). 1987;157:73-8
Pregnancy among obstetricians: a comparison of births before, during, and after residency (Grunebaum et al). 1987;157:79-83
A pregnant woman with a high level of naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii* (Konishi). 1987;157:832-3
Prescription drug use before and during pregnancy in a Medicaid population (Piper et al). 1987;157:148-56
The prevalence of autoantibodies in pregnant and nonpregnant women (Patton et al). 1987;157:1345-50
The reliability and clinical use of a rapid phosphatidylglycerol assay in normal and diabetic pregnancies (Saad et al). 1987;157:1516-20
Riboflavin concentration in maternal and cord blood in human pregnancy (Kirshenbaum et al). 1987;157:748-52
Updated pregnancy rates for the Today contraceptive sponge (Edelman and North). 1987;157:1164-5
Varicella zoster antibody testing in the care of pregnant women exposed to varicella (McGregor et al). 1987;157:281-4

Pregnancy in adolescence
Teen pregnancies (Roush) (Letter); (Hollingsworth) (Reply). 1987;157:1011

Pregnancy, ectopic; *see* **Ectopic pregnancy**

Pregnancy, multiple
Antenatal diagnosis and management of monoamniotic twins (Rodis et al). 1987;157:1255-7
The length of the human umbilical cord in twin pregnancies (Soernes and Bakke). 1987;157:1229-30
Umbilical cord waveforms in fetal surveillance of twins (Saldana et al). 1987;157:712-5

Pregnancy, prolonged; *see* **Postdate pregnancy**

Pregnancy complications; *see also* **Pregnancy**
Anorexia nervosa, bulimia, and pregnancy (Stewart et al). 1987;157:1194-8
Arachidonate lipoxygenase metabolites in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1454-60
Beneficial effects of U-63,557A, a thromboxane synthetase inhibitor, in an ovine model of pregnancy-induced hypertension (Keith et al). 1987;157:199-203
Blood pressure in the midtrimester and future eclampsia (Chesley and Sibai). 1987;157:1258-61
A classification of hypertension in pregnancy based on Doppler velocimetry (Ducey et al). 1987;157:680-5
Calculated capillary hydrostatic pressure in normal pregnancy and preeclampsia (Øian and Maltau). 1987;157:102-6
Cerebrospinal fluid levels of magnesium in patients with preeclampsia after treatment with intravenous magnesium sulfate: a preliminary report (Thurnau et al). 1987;157:1435-8
Colposcopic evaluation of abnormal Papanicolaou smears in pregnancy (Benedet et al). 1987;157:932-7
Disruption of embryonic and fetal development due to preimplantation chemical insults: a critical review (Iannaccone et al). 1987;157:476-84 (Curr. dev.)
Drug prescribing for thromboembolic disease during pregnancy (Greenspoon and Montoro) (Letter). 1987;157:784-5
Elevated maternal serum α-fetoprotein levels and oligohydramnios: poor prognosis for pregnancy outcome (Dyer et al). 1987;157:336-9
Factor XI deficiency presenting as hemarthrosis during pregnancy (Musclow et al). 1987;157:178-9
Fetal growth retardation as a complication of pregnancy in patients with neurofibromatosis (Blickstein and Lancet). 1987;157:343
First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions (Strobino and Pantel-Silverman). 1987;157:1150-4
Gestational diabetes: insulin requirements in pregnancy (Langer et al). 1987;157:669-75
Gestational diabetes: maternal response to diet and insulin therapy as reflected by glycosylated hemoglobin concentration (Grandis et al). 1987;157:1118-21
Haptoglobin helps diagnose the HELLP syndrome (Poldre). 1987;157:1267
Hereditary protein C deficiency during pregnancy (Brenner et al). 1987;157:1160-1

Human chorionic gonadotropin, estriol, and testosterone changes in two pregnancies with hyperreactio luteinalis (Muechler et al). 1987;157:1126-8
Hyperimmunoglobulinemia E and pregnancy: a case report (Lindenbaum et al). 1987;157:1273-4
Hypertensive diseases of pregnancy: nulliparity, primiparity, multiparity, and gravidity (Reamy) (Letter); (Gleicher) (Reply). 1987;157:1012
Increased fetoplacental active renin production in pregnancy-induced hypertension (Brar et al). 1987;157:363-7
Lymphocyte-reactive antibodies and recurrent early pregnancy failure (Biddle et al) (Letter). 1987;157:785-6
Mycotic aneurysms in pregnancy (Boike et al). 1987;157:340-1
The origin of increased serum iron in pregnancy-induced hypertension (Samuels et al). 1987;157:721-5
Pemphigus in pregnancy: a reevaluation of fetal risk (Metzker and Merlob) (Letter). 1987;157:1012-3
Postprandial walking exercise in pregnant insulin-dependent (type 1) diabetic women: reduction of plasma lipid levels but absence of a significant effect on glycemic control (Hollingsworth and Moore). 1987;157:1359-63
Pregnancy in Henoch-Schönlein purpura (Joseph et al). 1987;157:911-2
The pregnant brain dead and the fetus: Must we always try to wrest life from death? (Loewy). 1987;157:1097-101 (Clin. opinion)
A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor (Iams et al). 1987;157:638-43
Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1461-7
Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy (Schifman et al). 1987;157:304-7
Reduction of group B streptococcal maternal and neonatal infections in preterm pregnancies with premature rupture of membranes through a rapid identification test (Morales and Lim). 1987;157:13-6
The reliability and clinical use of a rapid phosphatidylglycerol assay in normal and diabetic pregnancies (Saad et al). 1987;157:1516-20
Renal arterial blood flow velocity waveforms in pregnant women (Hata et al). 1987;157:1269-71
Restriction fragment length polymorphisms of the insulin gene hypervariable region in gestational onset diabetes mellitus (Ober et al). 1987;157:1364-8
A screening test (GBS-Test) for urogenital carriage of group B streptococci (Christensen and Christensen). 1987;157:341-2
Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant (Druzin et al). 1987;157:1503-10
Soft tissue nuchal fold in the second-trimester fetus: standards for normal measurements compared with those in Down syndrome (Benacerraf and Frigoletto). 1987;157:1146-9
A thirty-year review of maternal mortality in Oklahoma, 1950 through 1979 (Moses et al). 1987;157:1189-94
The use of fetal biophysical profile improves pregnancy outcome in premature rupture of the membranes (Vintzileos et al). 1987;157:236-40

Pregnancy outcome; *see* **Outcome of pregnancy**

Pregnanediol
Natural family planning (Brown et al). 1987;157:1082-9

Pregnenolone
In vitro conversion of pregnenolone to progesterone in human term placenta and fetal membranes before and after onset of labor (Khan-Dawood). 1987;157:1333-7

Premature rupture of membranes; *see* **Fetal membranes**

Prematurity; *see also* **Preterm labor and delivery**
Genetic heterogeneity of prematurity and intrauterine growth retardation: clues from the Old Order Amish (Khoury and Cohen). 1987;157:400-10
Growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels compared in premature, small, average birth weight, and large infants (Samaan et al). 1987;157:1524-8
Regional program for prevention of premature birth in northwestern North Carolina (Meis et al). 1987;157:550-6

Prenatal care
Barriers to receiving adequate prenatal care (Poland et al). 1987;157:297-303
Prescription drug use before and during pregnancy in a Medicaid population (Piper et al). 1987;157:148-56

Prenatal diagnosis; *see also* **Screening**
Fetal cerebellar growth unaffected by intrauterine growth retardation: a new parameter for prenatal diagnosis (Reece et al). 1987;157:632-8
Soft tissue nuchal fold in the second-trimester fetus: standards for normal measurements compared with those in Down syndrome (Benacerraf and Frigoletto). 1987;157:1146-9

Preterm labor and delivery
Arachidonate lipoxygenase metabolites in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1454-60
Effects of the antiprogesterone RU 486 on preterm birth in the rat (Garfield et al). 1987;157:1281-5
Evaluation of 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: a new tocolytic agent (Hahn et al). 1987;157:977-82
Increased boy-to-girl ratio in women with preterm labor beginning with contractions (Seki and Kato) (Letter). 1987;157:215-6
The presence or absence of fetal breathing movements as a predictor of outcome in preterm labor (Besinger et al). 1987;157:753-7
Preterm birth at 23 to 26 weeks' gestation: Is active obstetric management justified? (Nwaesei et al). 1987;157:890-7
A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor (Iams et al). 1987;157:638-43
Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1461-7
Reduction of group B streptococcal maternal and neonatal infections in preterm pregnancies with premature rupture of membranes through a rapid identification test (Morales and Lim). 1987;157:13-6
Risk scoring for preterm labor: Where do we go from here? (Main and Gabbe). 1987;157:789-93 (Clin. opinion)
Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant (Druzin et al). 1987;157:1503-10

Ultrasonic prediction of birth weight in preterm fetuses: Which formula is best? (Pielet et al). 1987;157:1411-4

Prevention program

Regional program for prevention of premature birth in northwestern North Carolina (Meis et al). 1987;157:550-6

Progesterone

Effects of the antiprogesterone RU 486 on preterm birth in the rat (Garfield et al). 1987;157:1281-5

Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs (Elger et al). 1987;157:1065-74

Estrogen, progesterone, prolactin, prostaglandin E_2, prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ gradients across the uterus in women in labor and not in labor (Davidson et al). 1987;157:54-8

In vitro conversion of pregnenolone to progesterone in human term placenta and fetal membranes before and after onset of labor (Khan-Dawood). 1987;157:1333-7

Progesterone synthesis by human amnion, chorion, and decidua at term (Mitchell et al). 1987;157:349-53

Progestin

Continuous estrogen/progestin therapy in menopause (Prough et al). 1987;157:1449-53

Pituitary responses to synthetic corticotropin-releasing hormone: absence of modulatory effects by estrogen and progestin (Liu et al). 1987;157:1387-91

Progestogen; *see also* **Progesterone**

Coagulation effects of oral contraception (Bonnar). 1987;157:1042-8

Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs (Elger et al). 1987;157:1065-74

Metabolic effects of oral contraceptives (Gaspard). 1987;157:1029-41

New progestogens in oral contraceptives (Runnebaum and Rabe). 1987;157:1059-63

Progestogen potency in oral contraceptive pills (Gillmer). 1987;157:1048-52

Prolactin

Age-related changes in the female hormonal environment during reproductive life (Musey et al). 1987;157:312-7

Estrogen, progesterone, prolactin, prostaglandin E_2, prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ gradients across the uterus in women in labor and not in labor (Davidson et al). 1987;157:54-8

In vivo and in vitro modulation of growth hormone and prolactin of a mixed somatotropic-lactotropic pituitary microadenoma (Muechler et al). 1987;157:453-5

Modulating actions of estradiol on gonadotropin-releasing hormone–stimulated prolactin secretion in postmenopausal individuals (Christiansen et al). 1987;157:320-5

Promethazine

Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8

Proopiomelanocortin

Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: changes during the menstrual cycle (Petraglia et al). 1987;157:142-6

Propranolol

Effects of norepinephrine on lung fluid flow rate in the chronically catheterized fetal lamb (Higuchi et al). 1987;157:986-90

Propylthiouracil

Antithyroid drugs: to breast-feed or not to breast-feed (Cooper). 1987;157:234-5 (Clin. opinion)

Prostacyclin; *see also* **Prostaglandins**

Increased thromboxane biosynthesis in normal pregnancy is mainly derived from platelets (Fitzgerald et al). 1987;157:325-30

Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole (Wallenburg and Rotmans). 1987;157:1230-5

Prostaglandins; *see also* **Prostacyclin**

β-Adrenergic receptor agonist infusion increases plasma prostaglandin F levels in pregnant sheep (Casper and Lye). 1987;157:998-1003

Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs (Elger et al). 1987;157:1065-74

Estrogen, progesterone, prolactin, prostaglandin E_2, prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ gradients across the uterus in women in labor and not in labor (Davidson et al). 1987;157:54-8

A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)

Ovarian prostaglandin synthase: immunohistochemical localization in the rat (Curry et al). 1987;157:537-43

Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor (Romero et al). 1987;157:1461-7

Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua (Challis and Vaughan). 1987;157:1474-81

Temporal changes in uterine activity and prostaglandin response to RU 486 in rhesus macaques in late gestation (Haluska et al). 1987;157:1487-95

Treatment of polyhydramnios with prostaglandin synthetase inhibitor (indomethacin) (Cabrol et al). 1987;157:422-6

Protein

The effects of membrane permeability and binding by human serum proteins on steroid influx into the rabbit uterus (Steingold et al). 1987;157:1543-9

Hereditary protein C deficiency during pregnancy (Brenner et al). 1987;157:1160-1

Riboflavin concentration in maternal and cord blood in human pregnancy (Kirshenbaum et al). 1987;157:748-52

Seminal plasma proteins of fertile and infertile men analyzed by two-dimensional electrophoresis (Ayyagari et al). 1987;157:1528-33

Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short-and long-term metabolic control in gestational diabetes (Brustman et al). 1987;157:699-703

Proteolytic enzymes

The effects of proteolytic enzymes on in vitro ovulation in the rabbit (Yoshimura et al). 1987;157:468-75

Protoporphyrin test

Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy (Schifman et al). 1987;157:304-7

Pseudocholinesterase

Inhibitory effect of quinidine on plasma pseudocholinesterase activity in pregnant women (Kambam et al). 1987;157:897-9

Psychological adjustment; *see also* **Emotions**
Factors affecting psychological adjustment to a fetal death (Graham et al). 1987;157:254-7
Puerperium
Myocardial infarction in the puerperium (Feldman) (Letter); (Iffy et al) (Reply). 1987;157:515-6
Pulmonary hypoplasia
Fetal pulmonary hypoplasia with hydrothorax (Murayama et al). 1987;157:119-20
Pleural effusions and pulmonary hypoplasia (Castillo et al). 1987;157:1252-5
Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia (Johnson et al). 1987;157:764-9

Q

Quinidine
Inhibitory effect of quinidine on plasma pseudocholinesterase activity in pregnant women (Kambam et al). 1987;157:897-9

R

Race
Maternal serum α-fetoprotein screening. III. Pitfalls in evaluating black gravid women (Macri et al). 1987;157:820-2
Racial differences in survival of women with endometrial cancer (Bain et al). 1987;157:914-23
A thirty-year review of maternal mortality in Oklahoma, 1950 through 1979 (Moses et al). 1987;157:1189-94
Rapid identification test
Reduction of group B streptococcal maternal and neonatal infections in preterm pregnancies with premature rupture of membranes through a rapid identification test (Morales and Lim). 1987;157:13-6
Red cells
Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy (Schifman et al). 1987;157:304-7
Renal artery
Renal arterial blood flow velocity waveforms in pregnant women (Hata et al). 1987;157:1269-71
Renal function
The nonpredictive value of fetal urinary electrolytes: preliminary report of outcomes and correlation with pathologic diagnosis (Wilkins et al). 1987;157:694-8
Renin
Increased fetoplacental active renin production in pregnancy-induced hypertension (Brar et al). 1987;157:363-7
Residency
Pregnancy among obstetricians: a comparison of births before, during, and after residency (Grunebaum et al). 1987;157:79-83
Stress in residency: proposals for solution (Dilts and Dilts). 1987;157:1093-6 (Clin. opinion)
Respiratory distress syndrome; *see also* **Hyaline membrane disease**
Cholesteryl palmitate as a predictor of fetal lung maturity (Ludmir et al). 1987;157:84-8
Respiratory gases
Cord sampling for the evaluation of oxygenation and acid-base balance in human growth-retarded fetuses (Pardi et al). 1987;157:1221-8
Restriction fragment length polymorphism
Restriction fragment length polymorphisms of the insulin gene hypervariable region in gestational onset diabetes mellitus (Ober et al). 1987;157:1364-8
Resuscitation
A study on intrauterine fetal resuscitation with terbutaline (Patriarco et al). 1987;157:384-7
Rh isoimmunization
Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops (Socol et al). 1987;157:1369-75
Riboflavin
Riboflavin concentration in maternal and cord blood in human pregnancy (Kirshenbaum et al). 1987;157:748-52
Ringer's lactated solution
Prevention of pelvic adhesion formation by different modalities of treatment (Fayez and Schneider). 1987;157:1184-8
Risk
Hormonal contraception: benefits versus risks (Goldzieher). 1987;157:1023-8
Menstrual and reproductive factors and the risk of myocardial infarction in women under 55 years of age (La Vecchia et al). 1987;157:1108-12
Pemphigus in pregnancy: a reevaluation of fetal risk (Metzker and Merlob) (Letter). 1987;157:1012-3
A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor (Iams et al). 1987;157:638-43
Recurrent small for gestational age birth: perinatal risks and outcomes (Wolfe et al). 1987;157:288-93
Risk scoring for preterm labor: Where do we go from here? (Main and Gabbe). 1987;157:789-93 (Clin. opinion)
Status of infants at birth and risk for adverse neonatal events and long-term sequelae: a study in low birth weight infants (Luthy et al). 1987;157:676-9
Ritodrine
A randomized trial of ritodrine tocolysis versus expectant management in patients with premature rupture of membranes at 25 to 30 weeks of gestation (Garite et al). 1987;157:388-93
RU 486
Effects of the antiprogesterone RU 486 in normal women. I. Single-dose administration in the midluteal phase (Shoupe et al). 1987;157:1415-20
Effects of the antiprogesterone RU 486 in normal women. II. Administration in the late follicular phase (Shoupe et al). 1987;157:1421-6
Effects of the antiprogesterone RU 486 on preterm birth in the rat (Garfield et al). 1987;157:1281-5
Temporal changes in uterine activity and prostaglandin response to RU 486 in rhesus macaques in late gestation (Haluska et al). 1987;157:1487-95
Rupture of membranes; *see* **Fetal membranes**

S

Salpingostomy
Conservative surgical management of isthmic ectopic pregnancies (Smith et al). 1987;157:604-10
Scalp blade
Breakage of a fetal scalp blade with retention of fragments in the infant's scalp (Nieburg and Gross). 1987;157:441-2
Scalp blood
Intrapartum use of fetal scalp hematocrit in the diagnosis of profound fetal anemia caused by fetomaternal hemorrhage (Herman et al). 1987;157:1182-3

Scalp heat flux and its relationship to scalp blood pH of the fetus (Rudelstorfer et al). 1987;157:372-7
Validity of intrapartum diagnosis of fetal thrombocytopenia (Christiaens and Helmerhorst). 1987;157:864-5

Scalp heat flux
Scalp heat flux and its relationship to scalp blood pH of the fetus (Rudelstorfer et al). 1987;157:372-7

Screening
The "breakfast tolerance test": screening for gestational diabetes with a standardized mixed nutrient meal (Coustan et al). 1987;157:1113-7
Early third-trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term (Bochner et al). 1987;157:703-8
Fetal echocardiographic screening for congenital heart disease: the importance of the four-chamber view (Copel et al). 1987;157:648-55
Maternal serum α-fetoprotein screening: effects of weight adjustment (Kazazian et al) (Letter); (Macri et al) (Reply). 1987;157:782-4
Maternal serum α-fetoprotein screening. III. Pitfalls in evaluating black gravid women (Macri et al). 1987;157:820-2
Screening for genetic anomalies (Washburn) (Letter); (Macri) (Reply). 1987;157:212-3
A screening test (GBS-Test) for urogenital carriage of group B streptococci (Christensen and Christensen). 1987;157:341-2
A sonographic screening method for Down syndrome (Lockwood et al). 1987;157:803-8

Second-look surgery
Another look at the second-assessment procedure for ovarian epithelial carcinoma (Gallup et al). 1987;157:590-6

Seizures
Neonatal seizures after cesarean delivery: higher risk with labor (Spellacy et al). 1987;157:377-9
Spontaneous fetal seizures in utero (Maouris) (Letter). 1987;157:1009-10

Seminal fluid
Seminal plasma proteins of fertile and infertile men analyzed by two-dimensional electrophoresis (Ayyagari et al). 1987;157:1528-33

Sensitivity
Definitions of sensitivity, specificity, false negative results, and false positive results (Kolins) (Letter). 1987;157:517-8

Serum
Cord serum bromide concentration: variation and lack of association with pregnancy outcome (Miller et al). 1987;157:826-30
The effects of membrane permeability and binding by human serum proteins on steroid influx into the rabbit uterus (Steingold et al). 1987;157:1543-9
Elevated maternal serum α-fetoprotein levels and oligohydramnios: poor prognosis for pregnancy outcome (Dyer et al). 1987;157:336-9
Evaluation of serum CA 125 levels in the monitoring of ovarian cancer (Vergote et al). 1987;157:88-92
First-trimester serum levels of the β-subunit of human chorionic gonadotropin in a tubal molar pregnancy (Chase et al). 1987;157:910
Haptoglobin helps diagnose the HELLP syndrome (Poldre). 1987;157:1267
Maternal serum α-fetoprotein screening: effects of weight adjustment (Kazazian et al) (Letter); (Macri et al) (Reply). 1987;157:782-4
Maternal serum α-fetoprotein screening. III. Pitfalls in evaluating black gravid women (Macri et al). 1987;157:820-2
The origin of increased serum iron in pregnancy-induced hypertension (Samuels et al). 1987;157:721-5
Outcome in patients with unusually high maternal serum α-fetoprotein levels (Nelson et al). 1987;157:572-6
A pregnant woman with a high level of naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii* (Konishi). 1987;157:832-3
Serum CA-125 in the evaluation of benign adnexal cysts (Pittaway et al). 1987;157:1426-8
Serum copper level in gynecologic malignancies (Margalioth et al). 1987;157:93-6
Verified self-monitored blood glucose data versus glycosylated hemoglobin and glycosylated serum protein as a means of predicting short- and long-term metabolic control in gestational diabetes (Brustman et al). 1987;157:699-703

Sexual abuse
Configuration of the prepubertal hymen (Pokorny). 1987;157:950-6

Skin fibroblast
The binding and metabolism of low-density lipoprotein by skin fibroblasts of fetuses and newborns with anencephaly (Carr and Parker). 1987;157:1351-5

Small for gestational age
Recurrent small for gestational age birth: perinatal risks and outcomes (Wolfe et al). 1987;157:288-93

Smoking
Caffeine-cigarette interaction on fetal growth (Beaulac-Baillargeon and Desrosiers). 1987;157:1236-40
Cigarette smoking and the risk of female reproductive cancer (Stockwell and Lyman). 1987;157:35-40
The effect of smoking on placental and fetal zinc status (Kuhnert et al). 1987;157:1241-6
The relationship between cadmium, zinc, and birth weight in pregnant women who smoke (Kuhnert et al). 1987;157:1247-51

Society for Gynecologic Investigation
Papers of the Society for Gynecologic Investigation. 1987;157:1333-560

The Society of Perinatal Obstetricians
Transactions of the seventh annual meeting of The Society of Perinatal Obstetricians. 1987;157:627-781

Somatomedin C
Growth hormone, somatomedin C, and nonsuppressible insulin-like activity levels compared in premature, small, average birth weight, and large infants (Samaan et al). 1987;157:1524-8

Somatotropic-lactotropic pituitary microadenoma
In vivo and in vitro modulation of growth hormone and prolactin of a mixed somatotropic-lactotropic pituitary microadenoma (Muechler et al). 1987;157:453-5

Sonography; *see* **Ultrasound**

The South Atlantic Association of Obstetricians and Gynecologists
Transactions of the forty-ninth annual meeting of The South Atlantic Association of Obstetricians and Gynecologists. 1987;157:521-626

Specific gravity
Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes (Shinozuka et al). 1987;157:1140-5

Specificity
Definitions of sensitivity, specificity, false negative results, and false positive results (Kolins) (Letter). 1987;157:517-8

Spinal cord
The effect of severing the spinal cord of fetal lambs on length of gestation (Nowak et al). 1987;157:464-7
Squamous cell carcinoma
An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma (Senekjian et al). 1987;157:433-9
Statistics
When does a statistical fact become an ethical imperative? (Silver and Minogue). 1987;157:229-33 (Clin. opinion)
Sterilization
Contraception: an overview (Hammerstein). 1987;157:1020-3
Unintended laparotomy associated with laparoscopic tubal sterilization (Franks et al). 1987;157:1102-5
Steroids
The effects of membrane permeability and binding by human serum proteins on steroid influx into the rabbit uterus (Steingold et al). 1987;157:1543-9
Histologic composition of endometrial carcinomas analyzed for steroid receptor content (Soper et al). 1987;157:26-7
Human ovarian oxytocin and steroid hormones (Auletta and Flint) (Letter); (Khan-Dawood and Dawood) (Reply). 1987;157:1010-1
Management of preterm premature rupture of membranes: lack of a national consensus (Capeless and Mead). 1987;157:11-2
A new delivery system for contraceptive steroids (Segal). 1987;157:1090-2
Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua (Challis and Vaughan). 1987;157:1474-81
Stillbirth
Evaluation of a perinatal grief support team (Lake et al). 1987;157:1203-6
Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths (Manning et al). 1987;157:880-4
Strength conditioning
Effects of aerobic and strength conditioning on pregnancy outcomes (Hall and Kaufmann). 1987;157:1199-203
Streptococci
Effects of alterations of zinc-to-phosphorus ratios and meconium content on group B *Streptococcus* growth in human amniotic fluid in vitro (Hoskins et al). 1987;157:770-3
Reduction of group B streptococcal maternal and neonatal infections in preterm pregnancies with premature rupture of membranes through a rapid identification test (Morales and Lim). 1987;157:13-6
A screening test (GBS-Test) for urogenital carriage of group B streptococci (Christensen and Christensen). 1987;157:341-2
Stress
Stress in residency: proposals for solution (Dilts and Dilts). 1987;157:1093-6 (Clin. opinion)
Stress incontinence
Urogynecology: comments and caveats (Thiede and Saini). 1987;157:563-8
Stress test
A prospective comparative study of the extended nonstress test and the nipple stimulation contraction stress test (Devoe et al). 1987;157:531-7
A prospective evaluation of nipple stimulation techniques for contraction stress testing (Keegan et al). 1987;157:121-5
Stroke
Coagulation effects of oral contraception (Bonnar). 1987;157:1042-8
Stroma
The effect of insulin on aromatase activity in isolated human endometrial glands and stroma (Randolph et al). 1987;157:1534-9
Suture material
Careful surgical technique can reduce infectious morbidity after cesarean section (Lyon and Richardson). 1987;157:557-62
Symposium
International symposium on contraception. Part 1: oral contraception. 1987;157:1019-63
International symposium on contraception. Part 2: alternative methods of contraception. 1987;157:1064-92
symposium on infection: a complication of obstetric and gynecologic surgery. 1987;157:485-512
Systemic lupus erythematosus
Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant (Druzin et al). 1987;157:1503-10
Systolic/diastolic ratio
Uterine artery Doppler velocimetry: the significance of divergent systolic/diastolic ratios (Shulman et al). 1987;157:1539-42

T

Tachycardia
Mexiletine use in pregnancy and lactation (Lownes and Ives). 1987;157:446-7
Ultrasound evaluation of tachycardia-induced hydrops in the fetal lamb (Nimrod et al). 1987;157:655-9
Temperature
Careful surgical technique can reduce infectious morbidity after cesarean section (Lyon and Richardson). 1987;157:557-62
The normal parturient's admission temperature (Acker et al). 1987;157:308-11
Teratology
Disruption of embryonic and fetal development due to preimplantation chemical insults: a critical review (Iannaccone et al). 1987;157:476-84 (Curr. dev.)
Terbutaline
Chronic oral terbutaline tocolytic therapy is associated with maternal glucose intolerance (Main et al). 1987;157:644-7
A study on intrauterine fetal resuscitation with terbutaline (Patriarco et al). 1987;157:384-7
Term Guard uterine activity monitor
A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor (Iams et al). 1987;157:638-43
Testosterone
Human chorionic gonadotropin, estriol, and testosterone changes in two pregnancies with hyperreactio luteinalis (Muechler et al). 1987;157:1126-8
α-Thalassemia
Percutaneous ultrasound-guided fetal blood sampling in the management of nonimmune hydrops fetalis (Hsieh et al). 1987;157:44-9

Theca-lutein ovarian cysts associated with homozygous α-thalassemia (Miller et al). 1987;157:912-4

Thawing procedure
Human embryo viability related to freezing and thawing procedures (Testart et al). 1987;157:168-71

Theca-lutein ovarian cyst
Theca-lutein ovarian cysts associated with homozygous α-thalassemia (Miller et al). 1987;157:912-4

Thigh circumference
Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements (Vintzileos et al). 1987;157:410-4

Thoracic circumference
Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia (Johnson et al). 1987;157:764-9

Thrombocytopenia
Abnormalities in platelet antiglobulin tests in preeclamptic mothers and their neonates (Samuels et al). 1987;157:109-13
Haptoglobin helps diagnose the HELLP syndrome (Poldre). 1987;157:1267
Validity of intrapartum diagnosis of fetal thrombocytopenia (Christiaens and Helmerhorst). 1987;157:864-5

Thromboembolism; *see also* **Thrombosis**
Coagulation effects of oral contraception (Bonnar). 1987;157:1042-8
Drug prescribing for thromboembolic disease during pregnancy (Greenspoon and Montoro) (Letter). 1987;157:784-5

Thrombosis; *see also* **Thromboembolism**
Hereditary protein C deficiency during pregnancy (Brenner et al). 1987;157:1160-1
Venous thrombosis in patients with cardiac pacemakers and oral contraceptive use (Rao) (Letter). 1987;157:516-7

Thromboxane
Beneficial effects of U-63,557A, a thromboxane synthetase inhibitor, in an ovine model of pregnancy-induced hypertension (Keith et al). 1987;157:199-203
Increased thromboxane biosynthesis in normal pregnancy is mainly derived from platelets (Fitzgerald et al). 1987;157:325-30
Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole (Wallenburg and Rotmans). 1987;157:1230-5

Thyrotropin-releasing hormone
Transplacental stimulation of functional and morphologic fetal rabbit lung maturation: effect of thyrotropin-releasing hormone (Devaskar et al). 1987;157:460-4

Tocolysis
Chronic oral terbutaline tocolytic therapy is associated with maternal glucose intolerance (Main et al). 1987;157:644-7
Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes (Ducsay et al). 1987;157:1482-6
Evaluation of 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: a new tocolytic agent (Hahn et al). 1987;157:977-82
A randomized trial of ritodrine tocolysis versus expectant management in patients with premature rupture of membranes at 25 to 30 weeks of gestation (Garite et al). 1987;157:388-93

Today contraceptive sponge
Updated pregnancy rates for the Today contraceptive sponge (Edelman and North). 1987;157:1164-5

Toxemia; *see also* **Eclampsia; Preeclampsia**
Hereditary protein C deficiency during pregnancy (Brenner et al). 1987;157:1160-1

Toxoplasma gondii
A pregnant woman with a high level of naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii* (Konishi). 1987;157:832-3

Tracheal fluid
Drug accumulation in lung fluid of the fetal lamb after maternal or fetal administration (Riggs et al). 1987;157:1286-91

Training model
A reusable plastic training model for teaching Papanicolaou smear technique (Beckmann et al). 1987;157:259-60

Transabdominal needling
Transabdominal chorionic villus sampling: a freehand ultrasound-guided technique (Brambati et al). 1987;157:134-7

Transactions
Transactions of the forty-ninth annual meeting of The South Atlantic Association of Obstetricians and Gynecologists. 1987;157:521-626
Transactions of the seventh annual meeting of The Society of Perinatal Obstetricians. 1987;157:627-781

Transfusion
Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops (Socol et al). 1987;157:1369-75
Technical aspects of intravascular intrauterine transfusions: lessons learned from thirty-three procedures (Berkowitz et al). 1987;157:4-9 (Clin. opinion)

Translocation
A novel Y/13 familial translocation (Morris et al). 1987;157:857-8

Transport
Fetal monitoring during emergency obstetric transport (Elliott and Trujillo). 1987;157:245-7

Transurethral ultrasound
Transurethral ultrasound-guided oocyte retrieval for in vitro fertilization (Padilla et al). 1987;157:622-6

Trauma
Erb's palsy and fetal bruising after external cephalic version: case report (Petrikovsky et al). 1987;157:258
Fetal-induced trauma as a cause of antepartum hemorrhage (Eden). 1987;157:830-1

Trial of labor
Previous cesarean delivery and trial of labor (Fribourg) (Letter); (Finley) (Reply). 1987;157:1014-5
When does a statistical fact become an ethical imperative? (Silver and Minogue). 1987;157:229-33 (Clin. opinion)

Triphasics
Phasic approach to oral contraceptives (Hale). 1987;157:1052-8

Triploidy
Sonographic features of the triploid fetus (Lockwood et al). 1987;157:285-7

Trisomy 18
Genetic implications of idiopathic hydramnios (Landy et al). 1987;157:114-7

Trophoblast
HLA antigen expression and induction by γ-interferon in cultured human trophoblasts (Feinman et al). 1987;157:1429-34

Treatment of nonmetastatic gestational trophoblastic disease with oral methotrexate (Barter et al). 1987;157:1166-8

True hermaphrodite

The endocrinologic evaluation of a 45,X true hermaphrodite (Rosen et al). 1987;157:1272-3

Trypsin

Amniotic fluid trypsin and premature rupture of membranes (McGregor et al) (Letter); (Kanayama) (Reply). 1987;157:1316-9

Tubal molar pregnancy

First-trimester serum levels of the β-subunit of human chorionic gonadotropin in a tubal molar pregnancy (Chase et al). 1987;157:910

Tubal patency

Hysterosalpingographic evaluation of tubal patency after ectopic pregnancy (Mitchell et al). 1987;157:618-22

Tubal sterilization

Unintended laparotomy associated with laparoscopic tubal sterilization (Franks et al). 1987;157:1102-5

d-Tubocurarine

The use of fetal neuromuscular blockade during intrauterine procedures (Moise et al). 1987;157:874-9

Tubular enzyme

Increased tubular enzyme excretion in preeclampsia (Goren et al). 1987;157:906-8

Tumor; *see also* **Carcinoma; Neoplasia**

Diagnostic imaging of gynecologic tumors with the monoclonal antibody 791T/36 (Powell et al). 1987;157:28-34

An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma (Señekjian et al). 1987;157:433-9

Multiple primary gynecologic neoplasms (Rose et al). 1987;157:261-7

Serum copper level in gynecologic malignancies (Margalioth et al). 1987;157:93-6

Turner's syndrome

The endocrinologic evaluation of a 45,X true hermaphrodite (Rosen et al). 1987;157:1272-3

Twins

Antenatal diagnosis and management of monoamniotic twins (Rodis et al). 1987;157:1255-7

The length of the human umbilical cord in twin pregnancies (Soernes and Bakke). 1987;157:1229-30

Umbilical blood waveforms in fetal surveillance of twins (Saldana et al). 1987;157:712-5

U

U-63,557A

Beneficial effects of U-63,557A, a thromboxane synthetase inhibitor, in an ovine model of pregnancy-induced hypertension (Keith et al). 1987;157:199-203

Ultrasound

Abdominal pregnancy: magnetic resonance identification with ultrasonographic follow-up of placental involution (Spanta et al). 1987;157:887-9

Antenatal diagnosis with ultrasonography (Shalev and Zuckerman) (Letter). 1987;157:1323

The detection of facial anomalies with ultrasound (Koontz) (Letter); (Pilu) (Reply). 1987;157:1316

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Fetal-induced trauma as a cause of antepartum hemorrhage (Eden). 1987;157:830-1

Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes (Shinozuka et al). 1987;157:1140-5

The immediate effects of chorionic villus sampling on fetal movements (Boogert et al). 1987;157:137-9

In utero ponderal index as a prognostic factor in the evaluation of intrauterine growth retardation (Yagel et al). 1987;157:415-9

In vitro training model for diagnostic and therapeutic fetal intravascular needle puncture (Timor-Tritsch and Yeh). 1987;157:858-9

Outcome in patients with unusually high maternal serum α-fetoprotein levels (Nelson et al). 1987;157:572-6

Percutaneous ultrasound-guided fetal blood sampling in the management of nonimmune hydrops fetalis (Hsieh et al). 1987;157:44-9

Renal arterial blood flow velocity waveforms in pregnant women (Hata et al). 1987;157:1269-71

Soft tissue nuchal fold in the second-trimester fetus: standards for normal measurements compared with those in Down syndrome (Benacerraf and Frigoletto). 1987;157:1146-9

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Sonographic features of the triploid fetus (Lockwood et al). 1987;157:285-7

A sonographic screening method for Down syndrome (Lockwood et al). 1987;157:803-8

Transabdominal chorionic villus sampling: a freehand ultrasound-guided technique (Brambati et al). 1987;157:134-7

Transurethral ultrasound-guided oocyte retrieval for in vitro fertilization (Padilla et al). 1987;157:622-6

Ultrasonic prediction of birth weight in preterm fetuses: Which formula is best? (Pielet et al). 1987;157:1411-4

Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia (Johnson et al). 1987;157:764-9

Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage (Cardwell). 1987;157:358-9

Ultrasound evaluation of tachycardia-induced hydrops in the fetal lamb (Nimrod et al). 1987;157:655-9

Umbilical artery

Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow (Reed et al). 1987;157:774-9

Fetal umbilical artery flow velocimetry in postdate pregnancies (Guidetti et al). 1987;157:1521-3

Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation (Trudinger et al). 1987;157:1443-8

Umbilical artery flow velocity waveforms and the placental vascular bed (McCowan et al). 1987;157:900-2
Umbilical blood waveforms in fetal surveillance of twins (Saldana et al). 1987;157:712-5
Uterine and umbilical artery velocimetry during normal labor (Fleischer et al). 1987;157:40-3

Umbilical cord
Cord sampling for the evaluation of oxygenation and acid-base balance in human growth-retarded fetuses (Pardi et al). 1987;157:1221-8
The length of the human umbilical cord in twin pregnancies (Soernes and Bakke). 1987;157:1229-30
The relationships among the fetal biophysical profile, umbilical cord pH, and Apgar scores (Vintzileos et al). 1987;157:627-31
Umbilical cord blood pH and Apgar score as an index of neonatal health (Josten et al). 1987;157:843-8
Umbilical cord size and amniotic fluid volume in prolonged pregnancy (Silver et al). 1987;157:716-20
The umbilical cord twist: origin, direction, and relevance (Lacro et al). 1987;157:833-8

Umbilical transfusion
Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops (Socol et al). 1987;157:1369-75

Umbilical vein
Influence of umbilical vein administration of oxytocin on the third stage of labor: a randomized, double-blind, placebo-controlled study (Chestnut and Wilcox). 1987;157:160-2

Urethral closure pressure
Urogynecology: comments and caveats (Thiede and Saini). 1987;157:563-8

Urine electrolytes
The nonpredictive value of fetal urinary electrolytes: preliminary report of outcomes and correlation with pathologic diagnosis (Wilkins et al). 1987;157:694-8

Urodynamics
Urogynecology: comments and caveats (Thiede and Saini). 1987;157:563-8

Uterine artery
Uterine artery Doppler velocimetry: the significance of divergent systolic/diastolic ratios (Shulman et al). 1987;157:1539-42

Uterus
The cause of uterine softening in pregnancy (Jakobovits et al) (Letter). 1987;157:1013
The effects of membrane permeability and binding by human serum proteins on steroid influx into the rabbit uterus (Steingold et al). 1987;157:1543-9
Estrogen, progesterone, prolactin, prostaglandin E_2, prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ gradients across the uterus in women in labor and not in labor (Davidson et al). 1987;157:54-8
Evaluation of 1-deamino-[D-Try(Oethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: a new tocolytic agent (Hahn et al). 1987;157:977-82
A new look at uterine muscle contraction (Carsten and Miller). 1987;157:1303-15 (Curr. dev.)
A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor (Iams et al). 1987;157:638-43
Simultaneous objective and subjective evaluation of meclofenamate sodium in the treatment of primary dysmenorrhea (Smith and Powell). 1987;157:611-8
Uterine and umbilical artery velocimetry during normal labor (Fleischer et al). 1987;157:40-3

V

Vaccination; *see* **Immunization**

Vaginal bleeding
First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions (Strobino and Pantel-Silverman). 1987;157:1150-4

Vaginal hysterectomy
Single-dose prophylaxis for vaginal and abdominal hysterectomy (Hemsell et al). 1987;157:498-501

Vaginal mucus
Correlation between chlamydia infection and clinical evaluation, vaginal wet smear, and cervical swab test in female adolescents (Thejls et al). 1987;157:974-6
Preliminary observations on lactoferrin secretion in human vaginal mucus: variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae* (Cohen et al). 1987;157:1122-5

Vaporization
Laser excisional conization and laser vaporization for cervical intraepithelial neoplasia (Anderson) (Letter); (Baggish) (Reply). 1987;157:1322-3

Varicella zoster
Varicella zoster antibody testing in the care of pregnant women exposed to varicella (McGregor et al). 1987;157:281-4

Vascular lesions
Placental bed biopsy technique and vascular lesions (Labarrere) (Letter); (Robertson) (Reply). 1987;157:1320-2
A very rare fetal malformation: the cutaneous widespread vascular hamartomatosis (Arienzo et al). 1987;157:1162-3

Vasoconstriction
Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons (Howard et al). 1987;157:1261-6

Vasopressin
Circulating neurohypophyseal hormones in anencephalic infants (Oosterbaan and Swaab). 1987;157:117-9
The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation (Towell et al). 1987;157:1550-7
Vasopressin-induced bradycardia in fetal and adult sheep is not dependent on an increase in blood pressure (Harper and Rose). 1987;157:448-53

Vasotocin
Evaluation of 1-deamino-[D-Tyr(Oethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: a new tocolytic agent (Hahn et al). 1987;157:977-82

Velocimetry
A classification of hypertension in pregnancy based on Doppler velocimetry (Ducey et al). 1987;157:680-5
Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow (Reed et al). 1987;157:774-9
Fetal umbilical artery flow velocimetry in postdate pregnancies (Guidetti et al). 1987;157:1521-3

Renal arterial blood flow velocity waveforms in pregnant women (Hata et al). 1987;157:1269-71
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Umbilical blood waveforms in fetal surveillance of twins (Saldana et al). 1987;157:712-5
Uterine artery Doppler velocimetry: the significance of divergent systolic/diastolic ratios (Shulman et al). 1987;157:1539-42
Uterine and umbilical artery velocimetry during normal labor (Fleischer et al). 1987;157:40-3

Ventricular tachycardia
Mexiletine use in pregnancy and lactation (Lownes and Ives). 1987;157:446-7

Viability
Human embryo viability related to freezing and thawing procedures (Testart et al). 1987;157:168-71

Vibratory acoustic stimulation; *see* **Acoustic stimulation**

Virus; *see also* **Infection**
Isolation of acquired immunodeficiency syndrome virus from the placenta (Hill et al). 1987;157:10-1
Varicella zoster antibody testing in the care of pregnant women exposed to varicella (McGregor et al). 1987;157:281-4

Vulva
Necrotizing fasciitis of the vulva (Roberts). 1987;157:568-71

W

Walking
Postprandial walking exercise in pregnant insulin-dependent (type 1) diabetic women: reduction of plasma lipid levels but absence of a significant effect on glycemic control (Hollingsworth and Moore). 1987;157:1359-63

Waveforms; *see* **Velocimetry**

Weight
Fetal weight estimation formulas with head, abdominal, femur, and thigh circumference measurements (Vintzileos et al). 1987;157:410-4
Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes (Shinozuka et al). 1987;157:1140-5
Maternal serum α-fetoprotein screening: effects of weight adjustment (Kazazian et al) (Letter); (Macri et al) (Reply). 1987;157:782-4

Weight training
Effects of aerobic and strength conditioning on pregnancy outcomes (Hall and Kaufmann). 1987;157:1199-203

Wharton's jelly
Umbilical cord size and amniotic fluid volume in prolonged pregnancy (Silver et al). 1987;157:716-20

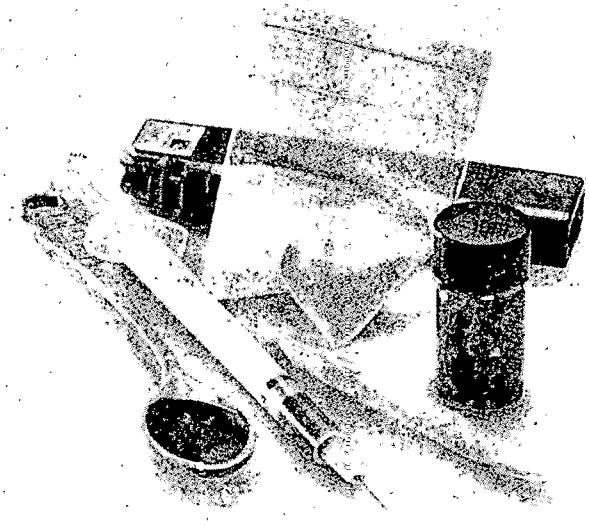
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Y chromosome
A novel Y/13 familial translocation (Morris et al). 1987;157:857-8

Z

Zinc
The effect of smoking on placental and fetal zinc status (Kuhnert et al). 1987;157:1241-6
Effects of alterations of zinc-to-phosphorus ratios and meconium content on Group B *Streptococcus* growth in human amniotic fluid in vitro (Hoskins et al). 1987;157:770-3
Magnesium and zinc status during the menstrual cycle (Deuster et al). 1987;157:964-8
Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy (Schifman et al). 1987;157:304-7
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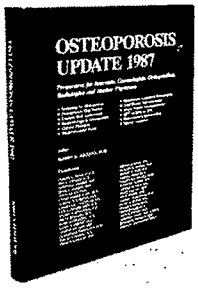
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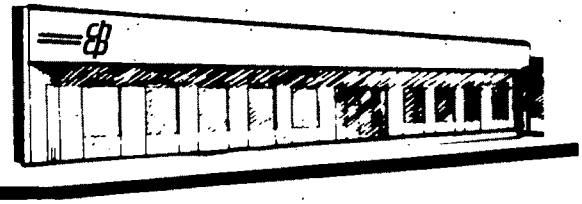
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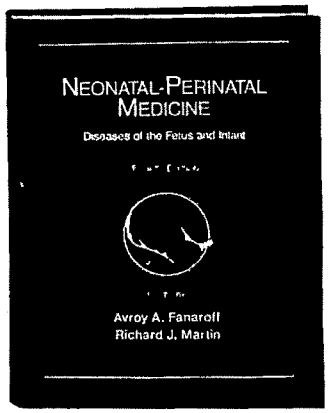
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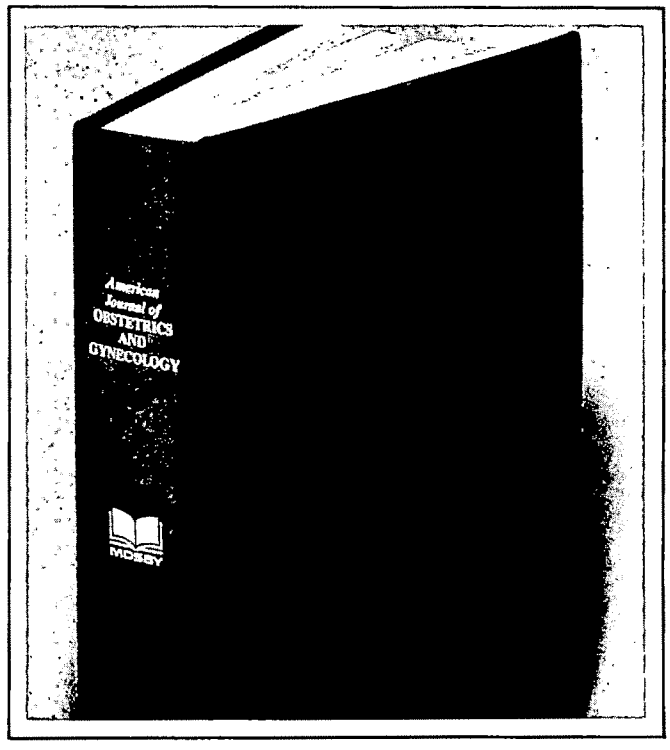
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INDEX TO ADVERTISERS

Akron City Hospital
Opportunity Available 40A
ASCCP
Meeting 28A
Billings Clinic
Opportunity Available 41A
Carle Clinic
Opportunity Available 42A
Catherine McAuley Health Center
Opportunity Available 42A
CHP Medical Group
Opportunity Available 41A
College of Physicians & Surgeons, The
Symposium 19A
Community Health Care Plan
Opportunity Available 41A
Cook County Graduate School of Medicine, The
Continuing Medical Education 24A
Erlanger Medical Center
Opportunity Available 39A
Filmtec, Inc.
Informed Consent Systems 28A
Flynn, Campbell, Collins' Executive Network
Opportunity Available 37A
Friedrich Group, The
Opportunity Available 39A
Gesco International, Inc.
Aspirator 24A
Hamad General Hospital
Opportunity Available 37A
Health Services Medical Corporation
Opportunity Available 38A
Hitchcock Clinic
Opportunity Available 37A
Kaiser Permanente
Opportunity Available 39A
Kaiser Permanente Medical Center
Opportunity Available 35A
Kaiser Permanente Medical Offices
Opportunity Available 41A
Marshfield Clinic
Opportunity Available 38A
Mary Imogene Bassett Hospital
Opportunity Available 38A
Maxicare Health Plans, Inc.
Recruitment 18A
Medical College of Georgia
Opportunity Available 37A
Medical College of Wisconsin
Opportunity Available 36A
Medical University of South Carolina
Opportunity Available 35A
Meeks, Rita Dr.
Opportunity Available 35A
Merck Sharp & Dohme
Mefoxin 25A,26A,27A,28A
Michael Reese Health Plan
Opportunity Available 36A
Mount Sinai Hospital Medical Center
Fellowship 40A
Multigon Industries, Inc.
Doppler 8A
Ortho Pharmaceutical Corporation
All-Flex 6A
Ortho-Novum 7/7/7 ..... Third Cover, Fourth Cover
Osler Institute, The
Symposium 46A
Permanente Medical Group, Inc.
Opportunity Available 36A,40A
Philadelphia Health Associates, P.C.
Opportunity Available 40A
Prime Health
Opportunity Available 35A
Quintessence Publishing Group
Symposium 19A
Radiology Postgraduate Education
Book 30A
Sea Mar Community Health
Opportunity Available 33A
St. Luke's-Roosevelt Hospital Center
Opportunity Available 34A
SUNY-Health Science Center
Fellowship 33A
Syntex Laboratories, Inc.
Femstat 14A,15A
Tri-Norinyl ..... Second Cover,1A,2A
Thomas, Charles C.
Books 32A
Trover Clinic
Opportunity Available 33A
University of Alabama
Opportunity Available 34A
University of Arizona
Opportunity Available 32A
University of California
Opportunity Available 32A
University of Health Sciences/The Chicago Medical School
Opportunity Available 34A
University of Massachusetts Medical Center
Opportunity Available 31A
University of Oklahoma
Fellowship 31A
University of Vermont
Opportunity Available 34A
University Physicians Foundation, Inc.
Opportunity Available 32A,33A
Upjohn Company, The
Provera 3A,4A
Vivgen Inc.
Lab Service 16A
Wright State University, The
Opportunity Available 30A

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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

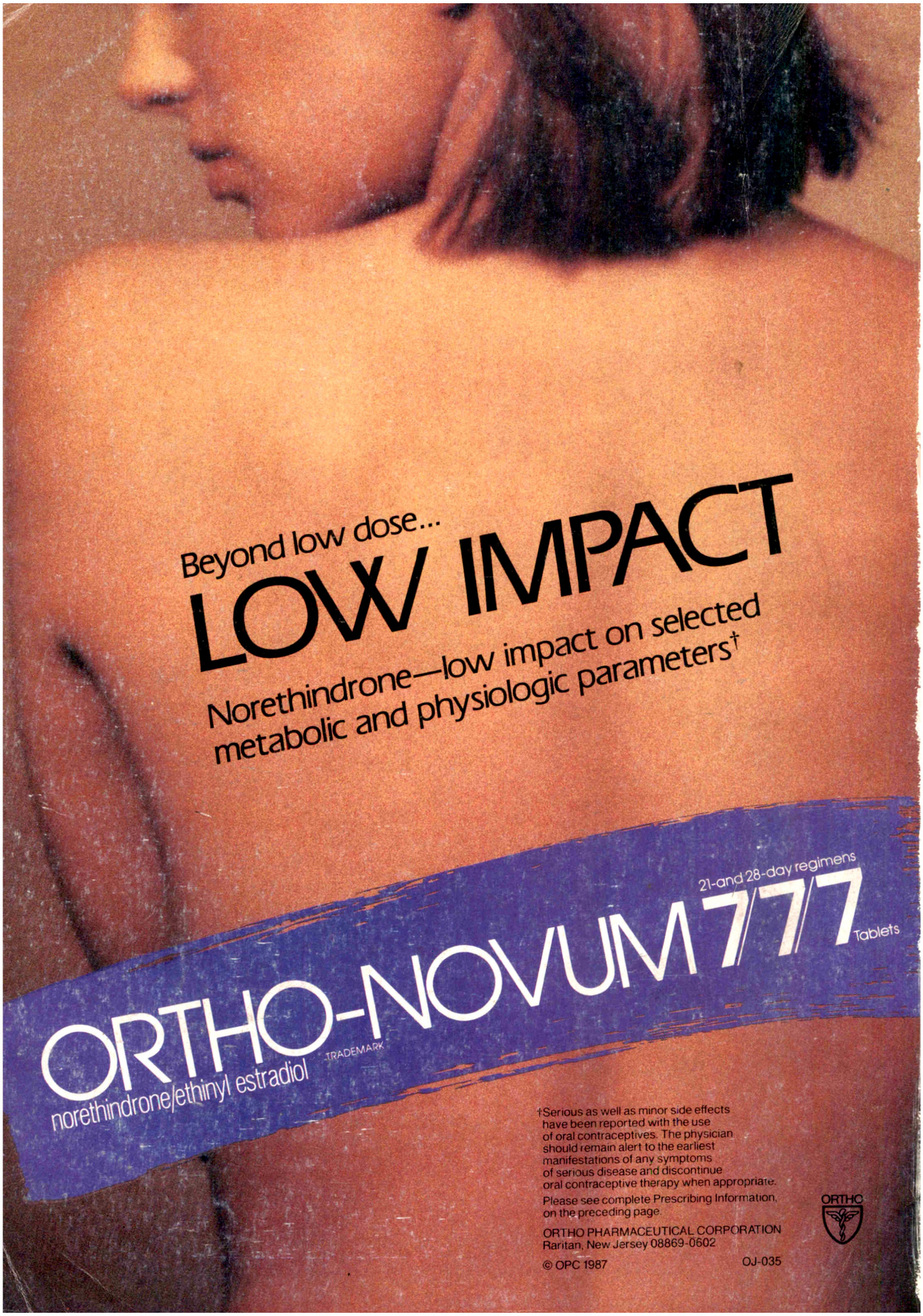
The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors or adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. Cases of hepatocellular carcinoma, although rare, have been reported in women taking oral contraceptives. Retrospective studies conducted in the United States and Britain report an increased risk of hepatocellular carcinoma in oral contraceptive users, particularly with increased duration of use. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **DRUG/LABORATORY TEST INTERACTIONS:** The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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Please see complete Prescribing Information on the preceding page.
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